

**COMPARISON OF CURRENT ALMOND PASTEURIZATION METHODS AND  
ELECTRON BEAM IRRADIATION AS AN ALTERNATIVE**

A Dissertation

by

MARY PIA CUERVO PLIEGO

Submitted to the Office of Graduate Studies of  
Texas A&M University  
in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

December 2011

Major Subject: Food Science and Technology

Comparison of Current Almond Pasteurization Methods and Electron Beam Irradiation  
as an Alternative

Copyright 2011 Mary Pia Cuervo Pliego

**COMPARISON OF CURRENT ALMOND PASTEURIZATION METHODS AND  
ELECTRON BEAM IRRADIATION AS AN ALTERNATIVE**

A Dissertation

by

MARY PIA CUERVO PLIEGO

Submitted to the Office of Graduate Studies of  
Texas A&M University  
in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

Approved by:

Chair of Committee,	Alejandro Castillo
Committee Members,	Rhonda Miller
	Rosana Moreira
	Mian Riaz

Intercollegiate Faculty Chair,	Alejandro Castillo
-----------------------------------	--------------------

December 2011

Major Subject: Food Science and Technology

**ABSTRACT**

Comparison of Current Almond Pasteurization Methods and Electron Beam Irradiation  
as an Alternative. (December 2011)

Mary Pia Cuervo Pliego, B.S., Instituto Tecnológico y de Estudios Superiores de  
Monterrey (México);

M.S., Texas A&M University

Chair of Advisory Committee: Dr. Alejandro Castillo

Two outbreaks of salmonellosis were linked to the consumption of raw California almonds in 2001 and 2004. Current federal regulations mandate that all almonds grown in California are to be treated with a process that results in a 4-log reduction of *Salmonella*. Since four out of the five approved technologies to pasteurize almonds rely on the application of heat to control *Salmonella*, the evaluation of alternative technologies against heat resistant *Salmonella* Senftenberg was imminent. In this study, almonds that were inoculated with *S. Enteritidis* PT 30 and *S. Senftenberg*, were treated with electron beam irradiation (e-beam), blanching and oil roasting. The thermal death time (D-value) for *S. Enteritidis* PT 30 when treated with e-beam was 0.90 kGy, 15 s when subjected to blanching at 88°C, and 13 s when treated with oil at 127 °C. Irradiation and thermal resistance of *S. Senftenberg* was not significantly different ( $P > 0.05$ ) from *S. Enteritidis* PT 30. The commercial application of e-beam as a pathogen intervention was assessed through Monte Carlo simulations (MCS) and experimental

measurements. The sensory characteristics of almonds commercially treated by e-beam, blanching and roasting were assessed by a consumer panel. Irradiated and blanched almonds did not differ in consumer overall like ( $P > 0.05$ ). Bitterness and rancidity attributes of irradiated almonds were between a “dislike slightly” and “dislike moderately”, whereas blanched and roasted almonds were between “neither like nor dislike” and “like slightly”. Almonds commercially irradiated, blanched and roasted were subjected to an accelerated shelf-life test (ASLT) evaluating percentage free fatty acids, peroxide value, and 2-thiobarbituric acid reactive substances (TBARs). No clear differences between treatments were observed at any given point in time in any of the chemical tests. A gas chromatography-mass-spectrometry-olfactometry (MDGC-MS-O) technology was used to compare full aroma and flavor profiles from raw and e-beam irradiated almonds. Differences in the aroma/odor profile and the taste analysis revealed that the difference between raw and irradiated almonds is extremely subtle. In conclusion, e-beam may be a feasible technology to control *Salmonella* in almonds if used at low doses, as a part of a series of interventions.

## **DEDICATION**

To my family, for teaching me that the sky is the limit. To Luis, for always being there...

## ACKNOWLEDGMENTS

I would like to thank my committee chair, Dr. Castillo, and my committee members, Dr. Miller, Dr. Moreira, and Dr. Riaz, for believing in me. I appreciate their guidance and support throughout the course of this research.

Special thanks to Dr. Kim for his invaluable technical expertise, and to Mr. Joe Maxim for sharing his creativity with me.

I want to express my gratitude to:

Mrs. Lisa Lucia, for taking me under her wing, and always leading me into the right direction.

My friends and mentors, Dr. Elisa Cabrera and Dr. Mayra Márquez, who inspired me to be strong and successful.

A very special family, the Richter's, for always being there, I will miss our long conversations.

All my friends and labmates, for their technical assistance and moral support throughout this journey, especially to Megha Adavi, Alex Brandt, Akhilesh Pandey, Keila Perez, Miguel Rojas, and Mariana Villarreal.

The National Council on Science and Technology of Mexico (CONACYT) for the economic support during most of my doctoral education.

Finally, I want to thank my family, for their constant encouragement and unconditional support that helped me pursue this dream.

## TABLE OF CONTENTS

	Page
ABSTRACT .....	iii
DEDICATION .....	v
ACKNOWLEDGMENTS.....	vi
TABLE OF CONTENTS .....	vii
LIST OF FIGURES.....	xi
LIST OF TABLES .....	xiv
INTRODUCTION.....	1
LITERATURE REVIEW .....	3
Almonds .....	3
History .....	3
Establishment of the Almond Board of California.....	3
Health benefits.....	4
World consumption .....	5
Agricultural production .....	5
Sources of pathogens.....	7
<i>Salmonella</i> and low moisture foods .....	10
Description of the microorganism.....	10
Salmonellosis .....	10
<i>Salmonella</i> in low <i>A<sub>w</sub></i> environments .....	13
Heat resistance of <i>Salmonella</i> in low <i>A<sub>w</sub></i> systems.....	14
Survival of <i>Salmonella</i> in low <i>A<sub>w</sub></i> foods .....	16
Mechanism for <i>Salmonella</i> 's increased heat tolerance at low <i>A<sub>w</sub></i> .....	17
Almonds and other low moisture foods as vehicles of <i>Salmonella</i> .....	22
Paprika and paprika powder .....	22
Peanut butter and peanut paste .....	23
Cocoa powder and chocolate.....	23
Almonds .....	24



	Page
Pistachio recall .....	25
Controlling <i>Salmonella</i> in almonds.....	26
Good agricultural practices as a prevention strategy.....	26
Interventions for pathogen control and current regulatory requirements for almonds.....	28
Heat treatments.....	29
Blanching .....	31
Oil roasting .....	32
Other thermal technologies .....	32
Non-thermal technologies .....	35
Electron beam irradiation as an intervention.....	37
Mode of action .....	38
Challenges to overcome in the irradiation of almonds.....	40
 MATERIALS AND METHODS .....	 43
General .....	43
Almond acquisition and storage.....	43
Bacterial cultures.....	43
Selective and differential media development .....	45
Preliminary studies.....	46
Growth characteristics.....	46
Thermal resistance of rifampicin resistant <i>Salmonella</i> strains.....	47
Radiation resistance of rifampicin resistant <i>Salmonella</i> strains.....	48
Restoration of almond Aw after storage and inoculation.....	49
Decimal reduction dose and time of <i>S. Enteritidis</i> rif+, and <i>S. Senftenberg</i> rif+ on inoculated almonds using electron beam irradiation, blanching, and oil roasting.....	51
Inoculum preparation .....	51
Bacterial inoculation and Aw restoration.....	52
Electron beam irradiation .....	54
Blanching .....	56
Oil roasting.....	57
Microbiological analysis .....	57

	Page
Establishment of the parameters required to achieve a 4-log reduction of a bacterial cocktail containing <i>S. Enteritidis</i> rif <sup>+</sup> , and <i>S. Senftenberg</i> rif <sup>+</sup> in a commercial electron beam irradiation facility.....	58
Monte Carlo simulation.....	58
Dose-mapping using alanine dosimeters.....	59
Evaluation of the chemical and sensory quality of almonds commercially processed with electron beam irradiation, blanching, and oil roasting .....	63
Irradiation of raw almonds .....	64
Sensory analysis .....	66
Gas chromatography and spectroscopy profile of raw and irradiated almonds .....	67
Quality of fatty acids .....	69
Oil extraction.....	69
Percentage of free fatty acids .....	70
Peroxide value .....	70
2-thiobarbituric acid reactive substances .....	71
Statistical analysis .....	72
<b>RESULTS AND DISCUSSION .....</b>	<b>74</b>
Preliminary studies.....	74
Growth characteristics.....	74
Thermal resistance of rifampicin resistant <i>Salmonella</i> strains.....	80
Radiation resistance of rifampicin resistant <i>Salmonella</i> strains.....	81
Restoration of almond Aw after storage and inoculation.....	81
Decimal reduction dose and time of <i>S. Enteritidis</i> rif <sup>+</sup> , and <i>S. Senftenberg</i> rif <sup>+</sup> on inoculated almonds using electron beam irradiation, blanching and oil roasting.....	82
Irradiation .....	82
Blanching .....	91
Oil roasting.....	93
Establishment of the parameters required to achieve a 4-log reduction of a bacterial cocktail containing <i>S. Enteritidis</i> rif <sup>+</sup> and <i>S. Senftenberg</i> rif <sup>+</sup> in a commercial electron beam irradiation facility .....	95
Monte Carlo simulation.....	95
Dose-mapping using alanine dosimeters.....	100

	Page
Evaluation of the chemical and sensory quality of almonds commercially processed with electron beam irradiation, blanching, and oil roasting .....	104
Sensory analysis .....	104
Oxidation of almond's lipid fraction .....	119
Percentage of free fatty acids .....	120
Peroxide value .....	122
2-thiobarbituric acid reactive substances .....	125
Gas chromatography and mass spectrometry profile of raw and irradiated almonds .....	127
<i>Aromatrax</i> ® results .....	137
CONCLUSIONS .....	143
REFERENCES .....	146
VITA .....	166

## LIST OF FIGURES

	Page
Figure 1 a) Alanine dosimeters inserted into almond kernel's flesh. b) Vertical stack of almond kernels with alanine dosimeters inserted in the flesh .....	60
Figure 2 Alanine dosimeters fixed in a horizontal, vertical and diagonal configuration inside a 12.7 x 12.7 x 12.7 cm cardboard box .....	61
Figure 3 Alanine dosimeters fixed in a horizontal, vertical and diagonal configuration inside a 12.7 x 12.7 x 12.7 cm cardboard box filled with almonds ....	62
Figure 4 Growth curves of <i>S. Enteritidis</i> PT 30 ATCC 1045 and <i>S. Enteritidis</i> rif <sup>+</sup> in tryptic soy broth at 37 °C .....	76
Figure 5 Growth curves of <i>S. Senftenberg</i> ATCC 43845 and <i>S. Senftenberg</i> rif <sup>+</sup> in tryptic soy broth at 37 °C.....	77
Figure 6 Three dimensional dose distribution of a horizontally placed almond kernel irradiated with a Van de Graaff accelerator of 1.35 MeV in a single beam configuration. Doses were estimated by Monte Carlo simulation .....	84
Figure 7 Three dimensional dose distribution of a horizontally placed almond kernel irradiated with a Van de Graaff accelerator of 1.35 MeV in a dual beam configuration. Doses were estimated by Monte Carlo simulation .....	85
Figure 8 Single (red and blue) and double sided (green) dose-depth curves of an almond kernel irradiated with a Van de Graaff accelerator of 1.35 MeV.....	87
Figure 9 Mean and standard deviations of the recovery of <i>S. Enteritidis</i> rif <sup>+</sup> and <i>S. Senftenberg</i> rif <sup>+</sup> from Nonpareil almonds irradiated at doses delivered with a Van de Graaff accelerator of 1.35 MeV .....	88
Figure 10 Mean and standard deviations of the recovery of <i>S. Enteritidis</i> rif <sup>+</sup> and <i>S. Senftenberg</i> rif <sup>+</sup> from Nonpareil almonds blanched at 88 °C at 6 time points.....	92
Figure 11 Mean and standard deviations of the recovery of <i>S. Enteritidis</i> rif <sup>+</sup> and <i>S. Senftenberg</i> rif <sup>+</sup> from Nonpareil almonds oil roasted at 127 °C at 6 time points.	94
Figure 12 CT scan of Nonpareil almonds packed in bulk. ....	97
Figure 13 Monte Carlo simulation of a box of almonds irradiated with an electron beam of 10 MeV on a single beam configuration .....	98

	Page
Figure 14 Monte Carlo simulation of a box of almonds irradiated with an electron beam of 10 MeV on a dual beam configuration.....	99
Figure 15 Horizontal placement of 6 alanine dosimeters fixed into a 12.7 cm <sup>3</sup> box filled with almonds and irradiated with a linear accelerator of 10 MeV in a dual beam configuration.....	101
Figure 16 Vertical assemble of 23 alanine dosimeters fixed into a 12.7 cm <sup>3</sup> box filled with almonds and irradiated with a linear accelerator of 10 MeV in a dual beam configuration.....	102
Figure 17 Diagonal assemble of 12 alanine dosimeters fixed into a 12.7 cm <sup>3</sup> box filled with almonds and irradiated with a linear accelerator of 10 MeV in a dual beam configuration.....	103
Figure 18 Ranking of attributes according to consumer's perceptions when consuming nuts.....	107
Figure 19 Consumers rankings according to their perceived level of acceptability of potential almond pasteurization technologies.....	109
Figure 20 Attributes evaluated on irradiated, blanched and roasted almonds with a 9-point-hedonic and intensity scales by a consumer panel.....	112
Figure 21 Consumer preference test. Consumers were asked to select their sample of choice between irradiated, blanched or roasted almonds for each attribute .....	117
Figure 22 Means and standard deviations of % free fatty acids of almonds commercially irradiated, blanched or roasted, and stored at 50°C and RH of 20% for 4 weeks .....	121
Figure 23 Means and standard deviations of peroxide values (meq/Kg) of almonds commercially treated by irradiation, blanching or roasting stored for 4 weeks at 50°C and RH of 20%.....	123
Figure 24 Means and standard deviations of 2-thiobarbituric acid compounds of almonds commercially treated by irradiation, blanching or roasting stored for 4 weeks at 50°C and RH of 20%.....	126
Figure 25 GC/MS Chromatograms of the raw (blue line) and irradiated almonds (black line).....	128

	Page
Figure 26 GC/MS Chromatograms of the raw (blue line) and irradiated almonds (black line) for the retention time window of 2-12 min .....	130
Figure 27 GC/MS Chromatograms of the raw (blue line) and irradiated almonds (black line) for the retention time window of 10-20 min .....	131
Figure 28 GC/MS Chromatograms of the raw (blue line) and irradiated almonds (black line) for the retention time window of 19.6-32 min .....	132
Figure 29 Aromagram <sup>®</sup> and chromatogram corresponding to raw almonds.....	139
Figure 30 Aromagram <sup>®</sup> and chromatogram of irradiated almonds.....	140

**LIST OF TABLES**

	Page
Table 1 Growth parameters for ATCC and rif+ <i>Salmonella</i> Enteritidis strains in tryptic soy broth at 37 °C .....	78
Table 2 Growth parameters for ATCC and rif+ <i>Salmonella</i> Senftenberg strains in tryptic soy broth at 37 °C .....	79
Table 3 Demographics of consumers participated in the sensory evaluation of commercially irradiated, blanched and roasted almonds .....	105
Table 4 Frequency of consumption of nuts and almonds by the consumers that participated in the sensory evaluation of commercially irradiated, blanched and roasted almonds .....	106
Table 5 Correlation of attributes rated by consumers that participated in the sensory evaluation of irradiated, blanched and roasted almonds .....	110
Table 6 Percentage of consumer ballots that included comments, and the classification of the comments for each type of almond processing .....	116
Table 7 Volatiles identified in irradiated samples by GC/MS and matched to previous reports .....	133
Table 8 Aroma profile of raw and irradiated almonds with the retention time, odor character, tentative identification and odor intensity for each aroma note .....	141

## INTRODUCTION

Every year, almond consumption keeps reaching all-time records in worldwide sales. Reports from the almond industry attribute the increased demand to higher crop availability and better consumer awareness of almonds health benefits (ABC 2009a). The principle of the Almond Board of California (ABC) of offering the best quality and safe product was at stake when almonds contaminated with *S. Enteritidis* were linked to two salmonellosis outbreaks in 2001 and 2004. As a result of the outbreaks, the ABC implemented an Action Plan requiring the pasteurization of almonds prior to reaching the consumers. This Action Plan led to the mandatory pasteurization of almonds regulation implemented since 2007 (ABC 2008). Most of the currently authorized pasteurization technologies rely only on heat as an intervention technology to control *Salmonella* (ABC 2010d). Therefore, the heat resistant strain *S. Senftenberg* has raised concerns in regards to the possible increased survival at the current heating critical limits. Research supporting the feasibility of thermal and non-thermal interventions to achieve the mandatory 4-log reduction of *Salmonella* is limited. The majority of the challenge studies aiming at obtaining a 4-log reduction of *Salmonella* in almonds have overlooked the increase of water activity ( $A_w$ ) resulting from the inoculation procedure. This might produce data that may be overestimating the power of the tested technology. Electron beam irradiation (e-beam) is a non-thermal technology that has been

---

This dissertation follows the style of Journal of Food Science.



extensively studied and used with the purpose of pathogen reduction in the food industry (FDA 2009). However, there are two challenges that e-beam irradiation has to overcome, quality deterioration and consumer acceptance. These two issues must be evaluated to complement feasibility studies of pathogen reduction interventions.

The objectives of the present study were: (a) to determine the decimal reduction dose and the thermal death time of *S. Enteritidis* PT 30 and *S. Senftenberg* on inoculated almonds, with a restored  $A_w$ , using electron beam irradiation, blanching and oil roasting; (b) to establish the parameters required to achieve a 4-log reduction of a bacterial cocktail containing *S. Enteritidis* PT 30 and *S. Senftenberg* in a commercial electron beam irradiation facility, and (c) to evaluate the chemical and sensory quality of almonds treated with electron beam irradiation, blanching and oil roasting commercially processed.

## LITERATURE REVIEW

### **Almonds**

#### *History*

Almonds (*Prunus amygdalus*) are recognized as indigenous to the desert region of western Asia and were brought to the European world by the Romans. In the 1850's, almond trees were brought to California by Spanish missionaries and orchards spread along the Central-Valley where the Mediterranean-like climate favored the crop (Rosengarten 1984, Kester and Ross 1996). Currently, an area of 299, 467 ha between Red Bluff and Bakersfield counties is exclusively dedicated to commercially grow almonds in California. The varieties most commonly cultivated are Nonpareil, Carmel and Butte (ABC 2009a).

#### *Establishment of the Almond Board of California*

The Almond Board of California (ABC) was established in 1950 under the federal marketing order No. 981. The Agricultural Marketing Service (AMS), of the U.S. Department of Agriculture (USDA) has administered the federal marketing order since its establishment. Because of this marketing order, almond growers and processors had heightened the almond industry through the establishment and implementation of quality regulations, research, and marketing projects (AMS 2010). The ABC is financially supported through an assessment on marketable kernel pound weight of almonds (AMS 2010). The ABC has sponsored numerous research projects which have stimulated the

development of the almond industry. Within the ABC, the Food Quality and Safety Committee ensures that California almonds are a safe and a high quality product. While seeking to provide a safe product, this committee has become a pioneer in the establishment and management of the currently mandated pasteurization of almonds (ABC 2008, AMS 2010).

### *Health benefits*

Numerous scientific studies have suggested the association of health benefits with almond consumption. According to these studies, almonds might help in reducing the risk of heart disease and hypercholesterolemia, to help in weight control, and cancer prevention. Recently, significant antioxidant capacity and prebiotic potential have been established (Mandalari and others 2008, Torabian and others 2009, Lapsley and Huang 2004, Phung and others 2009, Hollis and Mattes 2007).

In 2003, the International Tree Nut Council Nutrition Research and Education Foundation requested and was granted a qualified health claim from the U.S. Food and Drug Administration (FDA 2011). This claim allowed nut producers to exhibit the following statement: “Scientific evidence suggests, but does not prove that eating 1.5 ounces per d of most nuts, such as almonds, as part of a diet low in saturated fat and cholesterol may reduce the risk of heart disease”. It has been proposed that these health attributes are due to the almonds constituents, such as the mono and polyunsaturated fatty acids, soluble fiber, protein, plant sterols and flavonoids. Chung-Yen Chen and others (2006) and Torabian and others (2009) have emphasized that the interactions and

possible synergy among almonds nutrients, is a possible reason that leads to the well being of its regular consumers.

### *World consumption*

In 2007, more than 6,000 growers in California produced 1.36 billion lb of almonds, which accounts for 80% of the world production. Only 30% is consumed by the U.S market, and the remaining 70% is exported globally. Spain, Germany, India, Japan and China compose the top 5 export markets. Almond sales surpass the wine and wine products sales by more than a thousand million dollars in the specialty markets of California (Rosengarten 1984, Tolomeo and others 2008, ABC 2009a).

### *Agricultural production*

Almond trees go through a period of recovery and storage of nutrients from November to February. Following the dormancy stage, the warmer temperatures occurring in February and March trigger the growth of new blooms which prepare the trees for the next phase, pollination. The majority of the orchards in California cultivate almond varieties that are self-unfruitful, this means that the flower itself is unable to develop fruit with its own pollen. Therefore, flowers need to be cross-pollinated with another suitable variety. To accomplish an adequate pollination, honeybees are brought to the orchard as pollinator agents. It is a common practice to arrange trees in a variety-alternating-layout, such as, one row a major variety, followed by a row of a pollinizer variety. Pollinated flowers progress in the development of the kernel, which is

surrounded by a hard shell and hull. By July and August, the almonds have reached their ultimate size and the hull begins to dry and split. The nuts continue to dry on the trees and finally the hull opens completely. From mid-August through October, almonds are harvested using mechanical shakers. Tree trunk shakers are the most commonly used equipment to harvest almonds. Trees are shaken and the nuts fall onto the ground, where they are left to dry for one to two wk. Mechanical sweepers are used to blow the nuts piled around the trees in order to form stockpiles. Later, the stockpiles are lifted with a pickup machine and transported to a hulling plant for further processing. If rainfall and high relative humidity occurs at the time of harvest, almonds may not properly dry on the ground. These environmental conditions are usually found in Northern California, more so, than in the Central Valley. Should these conditions occur, an additional drying step is included before the hulls are removed at a hulling plant, as a step in the postharvest operations (Micke 1996, ABC 2010b).

Postharvest activities of almonds include cleaning, drying, hulling, shelling, sorting, packaging, and storage. At the hulling facility, almonds go through a pre-cleaning step, including vacuums, shaking screens, and gravity tables that are used to remove foreign materials, such as leaves, sticks, stones and dirt. Efficiency in the separation of the hull from the kernel is highly dependent on the moisture content of the nuts.

The most commonly utilized method to ensure that the moisture of the almonds is below 7% is the use of conveyor belts or batch hot air dryers. Deviations from this moisture specification may result in an increase of insect and mold infestation. Hulling

cylinders and shear rolls are used to remove the hulls from the in-shell almonds. Hulls are currently used as feed for dairy cattle. Shelling is carried out using counter-rotating cracking rolls and shells are separated using screens and blowers. Almond shells are sold commercially as bedding for livestock. Almond kernels are then sorted by color, size and defects. Screens, and more recently, electronic lasers, are used to grade product and to meet quality criteria of appearance and size. In some cases, for the highest quality markets, the last sorting is performed manually, by a line of qualified personnel. Finally, almonds are stored in bins under controlled temperature (4-7 °C) and relative humidity (< 65%) conditions (Micke 1996, ABC 2010b, Andrade 2010).

#### *Sources of pathogens*

During the growing and harvesting season, almonds can potentially become contaminated with pathogenic microorganisms present in the environment (ABC 2008). Uesugi and others (2007) performed drag-swabs in 17 almond orchard floors in California during a 5 year period. The drag-swab technique is a type of sampling commonly used to perform environmental tests. In general, the swab is made out of sterile rolled gauze pads, moistened in double-strength skim milk to improve *Salmonella* recovery (Kingston 1981, Byrd and others 1997). In Uesugi and others (2007) study, environmental samples were collected using a swab moistened in full-strength skim milk. The swab was dragged on the floor of the orchard, ensuring that the person sampling walked parallel to the swab. Of 228 samples tested, *Salmonella* was recovered from 53 samples (23%). Positive samples were most often seen from August to

December, coinciding with the time of harvest and post-harvest. The authors highlighted the increased use of machinery during the harvesting season, which increased the amount of dust and the possible spread of the surface-layer soil. The 53 *Salmonella* isolates were classified as *S. Enteritidis* PT 30, indicating the lasting persistence of this strain in the almond orchard soils. The highest percentage of positive samples on a single sampling day was recorded on October 19, 2004 (87% positives). These samples were collected in the middle a rain storm, after water had pooled on the orchard floor. The California Irrigation Management Information System (CIMIS) reported 20.8 mm of precipitations with average temperatures of 13 °C. The authors suggested that the higher number of *Salmonella*-positive samples on this specific day may have been the result of growth of the pathogen in the stack of wet almonds. Danyluk and others (2008b) studied the survival of *S. Enteritidis* PT 30 in artificially inoculated almond orchard soils. *S. Enteritidis* PT 30 was inoculated into two types of soil, Cereni and Milham, and samples were stored at 20 °C for 180 d. *S. Enteritidis* PT 30 was significantly reduced with time, Despite the significant reduction in population, *Salmonella* was still recovered from 88% of the samples after the 180 d incubation period. Additionally, the authors investigated the nutritional effect of sugars present in almond hulls as they lixiviate to the soil when rainfall or irrigation occurred. *S. Enteritidis* PT 30 was able to use the carbohydrates from hulls-water-extracts to promote its growth after 8 or 24 h of the addition of the extract (Danyluk and others 2008b). Uesugi and Harris (2006) found that after 48 h of inoculating *S. Enteritidis* PT 30 on almond hulls or shell slurries and incubating them at 24 °C, the pathogen was able to grow by approximately 4 and 3 log cycles, respectively.

Drying of almond hulls at 15 or 37 °C resulted in a reduction of 1 to 3 log CFU/g. However, as the incubation time increased, the reduction rate decreased. As previously mentioned, almonds are shaken from the trees and left on the ground for up to two wk before they are swept and processed (Micke 1996). Therefore, almonds are at risk of being contaminated while on the ground and spreading that contamination throughout the processing facility. Danyluk and others (2008a) evaluated the ability of environmental *S. Enteritidis* PT 30 to internalize into the almond kernel. The exterior surface of 5 varieties of almonds shells were soaked in a *S. Enteritidis* PT 30 suspension for 24 h at 24 °C. The pathogen of concern was isolated from the inner surface of almond shells regardless of the variety. Uesugi and others (2006) assessed the survival of *S. Enteritidis* PT 30 on inoculated almonds after 161 d at 23 °C. Reduction rates were not significantly different among inoculum levels, from 1 to 8 log CFU/almond. Almonds inoculated at 8 log CFU/almond were further stored at -20 °C and 4 °C for 550 d, and no significant reductions were observed. On the other hand, when the same inoculated almonds were stored at 23 °C, a reduction rate of 0.30 log CFU/month was achieved after 550 d of storage. Danyluk and others (2007) determined the prevalence of *Salmonella* from California raw almonds collected from seven almond handlers over a 5 year period. A prevalence of 0.87% was estimated with levels between 1.2 and 2.9 MPN/100 g, and 35 different serotypes were involved.

These previous studies confirm the chronic prevalence, survival, and possible conditions for growth of *Salmonella* at the orchard floor. Additionally, the internalization of the pathogen through the shell allowing the contamination of the



almond kernel, has been well established. Finally, *Salmonella* demonstrated the ability to survive for extended periods of time on the surface of almond kernels. In conclusion, *Salmonella* is a hazard reasonably likely to be present in raw almonds and it may cause disease if it is not effectively controlled.

### ***Salmonella* and low moisture foods**

#### *Description of the microorganism*

The genus *Salmonella* includes facultatively anaerobic, non-sporeforming Gram negative rods, and belongs to the *Enterobacteriaceae* family. Several taxonomic schemes for the classification of the more than 2400 serovars of *Salmonella* have been developed over the last 50 years. The currently used scheme, which is based on DNA – DNA hybridization and multilocus enzyme electrophoretic characterization, identifies only two species, *S. enterica* and *S. bongori*. Within *S. enterica* there are 6 subspecies; *enterica*, *salamae*, *arizonae*, *diarizonae*, *houtenae*, and *indica* (D’Aoust 1997, D’Aoust 2000, Jay 2005). *Salmonella* is part of the natural intestinal microflora of several birds, mammals, reptiles and insects, which contributes to the wide-spread nature of this pathogen (D’Aoust 2000).

#### *Salmonellosis*

The Centers for Disease and Control and Prevention (CDC 2009) has estimated that in the U.S. 400,000 cases of salmonellosis occur annually, and this number of infections appears to be on the rise. The national surveillance report from 1998 to 2002

showed that *Salmonella* serotype Enteritidis accounted for the largest number of outbreaks and outbreak-related cases in the U.S. In the document “Surveillance for Food borne-Disease Outbreaks --- United States, 1998-2002” published by the Morbidity and Mortality Weekly Report (MMWR) eggs and poultry had the highest incidence of *Salmonella* compared to any other commodity. In a more recent report (2002-2005) by the Food Safety and Inspection Service (FSIS) the incidence of *Salmonella* in poultry appears to be declining. Similarly, routine testing performed by the FSIS at slaughter and meat processing plants has shown a decrease in the contamination of ground beef by *Salmonella* since 1998. *Salmonella* spp. infections are usually related to animal origin products, such as meat and eggs, however, fresh produce and nuts have been recently implicated in numerous outbreaks (Isaacs and others 2005, CDC 2010a).

Common symptoms of salmonellosis present an onset between 8 to 72 h and may include nausea, severe abdominal cramps, diarrhea, vomiting, and fever (Jay 2005, FDA 2010a). Salmonellae infectious dose was once believed to be  $10^5$  to  $10^7$  cells, but more recent literature reports 15 to 20 cells or as few as a single cell, depending upon the individual and the strain (D’Aoust 2000, FDA 2010a). Enterocolitis is usually self-limiting and ceases within 4 to 5 d. Severe cases of diarrhea can be treated with electrolyte replacements (FDA 2010a). In cases where the salmonellae travel into deeper tissues, through vascular and lymphatic channels, the infection can deteriorate into septicemia. Chronic consequences such as aseptic reactive arthritis, Reiter's syndrome, ankylosing spondylitis, and rheumatoid arthritis, may follow 3 to 4 w after the onset the of disease (D’Aoust 2000).

*Salmonella*'s invasion mode has been studied for more than 40 years, and the scientific community is still deciphering the convoluted mechanism. One of the first documents describing the internalization of the *Salmonella* cell into the epithelial lining of the small intestine was done by Takeuchi (1966). Recent studies have aimed to understand the molecular and gene regulation pathways involved in the invasion of epithelial cells by *Salmonella* (Galán 2001). *S. Enterica* carries in its chromosome pathogenicity islands I and II (SPI-1, SPI-2) (Wallis and Galyov 2000, Jay 2005). Within the SPIs there are two Type III Secretions Systems (TTSSs) that are essential for pathogenicity. Most of the virulence genes are loaded into the SPIs (Galán 2001). TTSS-1 secretes proteins that are relevant to the invasion of epithelial cells, whereas TTSS-2 is required for systemic infection (Wallis and Galyov 2000, Galán 2001, Ohl and Miller 2001).

*Salmonella* infection starts with the pathogen colonizing the mucosal enterocytes (D'Aoust 2000, Jones and Falkow 1996). The initial attachment to the intestinal mucosa is achieved with the collaboration of fimbrial adhesins encoded in the SPI-1 (Galán 2001). Invasion into the epithelial cells follows a bacterial-mediated endocytosis model. The infection site seems to be dependent on host-pathogen interactions. Studies in mice have found that salmonellae are more inclined to adhere to the microfold cells (M cells) of the intestinal epithelium, whereas in bovine systems, the M cells are apparently not the preferred site of infection (Ohl and Miller 2001).

Soon after the pathogen has attached to the epithelial surface, drastic changes in the cytoskeleton configuration of the host take place. Subsequent disruption of the

normal epithelial brush and the formation of ruffles in the membranes of M cells and enterocytes occur (Ohl and Miller 2001, Wallis and Galyov 2000). Finally, *Salmonella* is engulfed into membrane bound vesicles by macropinocytosis, where it resides and multiplies until the cells explode and the infection is spread (Wallis and Galyov 2000, Jay 2005)

Once the pathogen has invaded the cytoplasm of epithelial cells, the immune system elicits a flood of polymorphonuclear leukocytes (PMN) to the infected area. These leucocytes release prostaglandin, which results in mucosal inflammation. Within the inflamed tissue, *Salmonella* releases an enterotoxin that leads to the activation of adenyl cyclase, resulting in watery diarrhea (Jay 2005, D'Aoust 2000, Darwin and Miller 1999).

#### *Salmonella in low $A_w$ environments*

Water activity represents the degree of interaction between the water and the rest of the nonaqueous constituents in a food product. It is also described as the amount of water available for chemical or microbiological reactions. Water that is strongly bound to solutes becomes unavailable for chemical reactions or microbial growth (Fennema 2000). This is the reason why it is possible to extend the shelf-life of a food product by eliminating or binding its water (Fennema 2000, Jay 2005). Numerically,  $A_w$  is defined as the ratio between the vapor pressure of the food divided by the vapor pressure of pure water, both at the same temperature. Hence, it is a dimensionless parameter ranging from

0 to 1.0, in pure water. A food product with a  $A_w$  below 0.6 is considered low moisture (Fennema 2000, Jay 2005).

Pistachios, peanut butter, paprika, cocoa powder, chocolate, and almonds are considered low moisture food. These food items have recently been implicated in recalls and outbreaks of *Salmonella* (CDC 2010b). The relevance of these recalls and outbreaks lies on the fact that *Salmonella* is able to survive in low  $A_w$  products, and has an increased heat-resistance in low  $A_w$  products (Lehmacher 1995, D'Aoust 2000, CDC 2010b). Abundant literature supports the increased heat resistance and extended survival of *Salmonella* when osmotically challenged.

#### *Heat resistance of Salmonella in low $A_w$ systems*

Riemann (1968) studied the heat resistance of *S. Typhimurium* and *S. Senftenberg 775W* inoculated on meat and bone meal with adjusted  $A_w$ . Results showed that *S. Typhimurium* was more heat resistant than *S. Senftenberg 775W*. The highest reduction (6 log cycles) was achieved in samples with  $A_w$  of 0.88 heated at 90 °C for 20 min. These results highly contrasts with the 3 log reduction found in samples with a  $A_w$  of 0.62, which were heated at 100 °C for the same length of time. Inoculated samples with  $A_w$  of 0.9 that were kept at room temperature for 6 wk had reductions between 5 to 6 log cycles. However, the application of such conditions as a decontamination step was challenged by the author, due to the risk of mold infestation when product is held at such high  $A_w$ . McDonough and Hargrove (1968) studied the survival of a cocktail of *S. Senftenberg 775 W*, *S. Typhimurium TM1* and *S. Newbrunswick 1608* when artificially

inoculated into nonfat dry milk (NFDM). A thin layer heating treatment of 3 min at 115.5 °C reduced 0.9 and 1.5 logs at 4 and 25% moisture, respectively. Additionally, NFDM was reconstituted to 10 and 50% solids and heated to 65.5 °C for 1 min. The reduction of *Salmonella* as a result of this heat treatment was inversely related to the solid concentration. In the fluid milk with the higher percentage of solids, there was a reduction of approximately 4 log cycles, whereas the reduction in the milk with the lower percentage of solids was of approximately 6 log cycles. Liu and others (1969) investigated the heat resistance of *S. Senftenberg 775W* in meat and bone meal and in chick starter at moisture contents from 5 to 30%. The thermal resistance decreased as feed moisture increased. While maintaining the percent moisture constant, the heat resistance varied on the food type. The D-value was greater in meat and bone meal than in chick starter at all moisture levels. Goepfert and others (1970) observed an increase in the heat resistance of *Salmonella* and *Escherichia coli* when the  $A_w$  of the medium decreased. The  $A_w$  in the test medium was adjusted by adding sucrose, fructose, glycerol or sorbitol, finding that sucrose conferred a higher degree of heat protection compared to the other solutes. Differences between strains were observed as well. Microorganisms that were osmotically shocked in a glycerol solution at the growth stage, showed a greater heat resistance. Similarly, Corry (1974) found that the heat resistance of 3 strains of *Salmonella* was increased as the concentration of solutes in the medium increased. Some solutes showed greater protective effect against heat than others. The order of the solutes from more protective to least protective was: sucrose, glucose, sorbitol, fructose and glycerol. More recently, Mattick and others (2001)

studied the heat tolerance of *S. Typhimurium* DT104. In this study, broths were supplemented with appropriate amounts of a mixture of glucose-fructose to adjust the  $A_w$ . There were 54 treatments, including 9 challenge temperatures, (55–80 °C), and 6  $A_w$  (0.65-0.90). Results showed that low  $A_w$  was unfavorable to survival at 55 or 60 °C, while treatments at 70 °C or higher, were less lethal as the  $A_w$  dropped.

The increased heat resistance of *Salmonella* at low  $A_w$ , has also being documented in inoculated food matrixes. Doesburg and others (1970) artificially inoculated fishmeal with *S. Oranienburg*, and *S. Senftenberg* 775W to which heat treatments ranging from 50 to 85 °C were applied. Death rates increased when  $A_w$  was reduced below 0.58. *S. Oranienburg* heat resistance was greater than *S. Senftenberg* 775W. In the same study, *S. Senftenberg* 775W showed a maximum heat resistance at  $A_w$  of 0.53. Shachar and Yaron (2006) reviewed the heat tolerance of a *S. Agona*, *S. Enteritidis* and *S. Typhimurium* inoculated in peanut butter. Heat treatments at 70, 80 and 90 °C were applied for 50 min. Initial inoculum levels were 8 log CFU/g of peanut butter. Differences between serovars were not identified. Treatment at 70 °C had a 2.7 log CFU/g reduction, whereas treatments at 80 and 90 °C reached a 3.0 log CFU/g reduction.

#### *Survival of Salmonella in low Aw foods*

Beuchat and Heaton (1975) explored the survival of *S. Senftenberg* 775W, *S. Anatum* and *S. Typhimurium* inoculated onto inshell Stuart pecans with a moisture level of 11.5%. Survival was evaluated throughout a 32 week period at -18, -7, 5 and 21 °C.

After 16 wk at 21 °C, populations of *S. Senftenberg* 775W and *S. Anatum* were reduced approximately 3 and 4 logs, respectively. Storage temperature was inversely proportional to *Salmonella* survival. Nuts stored at -18 °C for 32 wk only presented minor reductions in viable salmonellae. Tamminga and others (1977) compared the survival of *S. Typhimurium* and *S. Eastbourne* when inoculated into chocolate bars by two inoculation methods; direct inoculation on the chocolate bar, and inoculation of the milk powder used as an ingredient in the chocolate preparation. *Salmonella* populations declined by approximately 3 log cycles through a period of 17 mo. *S. Typhimurium* population had a steeper decline than *S. Eastbourne*. *S. Eastbourne* had a higher survival rate when inoculated into the milk powder compared to direct inoculation into the chocolate bar. Juven and others (1984) investigated the survival of *S. Montevideo* and *S. Heidelberg* inoculated into dry milk, cocoa powder, poultry feed and meat and bone meal at  $A_w$  from 0.4 to 0.75. These authors found that *S. Montevideo* had higher survival rates than *S. Heidelberg*. *Salmonella* recovery was similar between  $A_w$  0.43 and 0.52, but greater than at 0.75.

#### *Mechanism for Salmonella's increased heat tolerance at low $A_w$*

The cross-protection between osmotic stress and the increased thermal resistance of *Salmonella* has been documented more than 50 years ago. Recent studies now focus on the reasons behind the relatedness of osmotic stress and increased heat tolerance.

In a bacterial cell under stress-free conditions, the cytoplasmic membrane applies turgor pressure to the cell wall, due to the  $A_w$  differential between the inner cell and the



surrounding environment (Bianchi and Baneyx 1999). According to these authors, if the Aw of the matrix is reduced, osmotic shock causes the release of water and loss of turgor, which can lead to cell disruption and consequent death (Bianchi and Baneyx 1999). According to Csonka (1981), bacteria's internal osmolarity is achieved by the accumulation of inorganic ions and amino acids inside the cell. Proline was one of the first compounds reported to act as an osmotic balancer.

When microorganisms are exposed to high, but not-lethal temperatures they acquire a protection that allows them to withstand further treatments at temperatures that were originally lethal. Habituation to a sublethal stresses can induce tolerance to more extreme stresses leading to cross-protection to other stresses. This protection is conferred by the production of a number of proteins, such as chaperonins and proteases (Fletcher and Csonka 1998, Mattick and others 2000b).

Chaperons are proteins that aid in the folding, and transportation of other newly synthesized proteins. Newly formed polypeptide chains need to undergo a folding process to become a three dimensional actively functional protein (Georgopoulos 1992). Hsp (Heat shock protein) 60, or chaperonins, are a type of bacterial chaperones. Hsp 60, binds to chains of amino acids as soon as they are released from the ribosome. Unfolded proteins are captured by chaperonins subunits through a series of hydrophobic interactions before folding occurs in the central ring cavity of the chaperonin. Inside the chaperonin structure, the newly formed chain of amino acids is protected from aggregating with other nonnative proteins. The mechanism of binding and releasing a folded protein is ATP regulated (Georgopoulos 1992, Hartl and Hayer-Hartl 2002).

Fletcher and Csonka (1998) and Fletcher and others (2001) challenged the resistance of *S. Typhimurium* LT2 to heat (50 °C) and oxidizing agents hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>). Numbers of survivors increased when the solution contained 0.3 M of NaCl, however, the effect receded when glycine betaine was added to the medium. The fact that thermal and oxidizing resistance dropped after the addition of glycine betaine might suggest that both stresses were repressed by a common stress-response mechanism. The elimination of the thermal resistance by the addition of glycine betaine was simultaneous to the down-regulation of genes recognized to aid in osmotic adaptation mechanisms (Fletcher and others 2001). According to Gunasekera and others (2008) prokaryotic cells contain a collection of transcriptionally regulated genes which prepare them to resist non-favorable environmental factors, such as Aw, pH, temperature and oxygen concentration. These genes are commonly referred as stress or shock response systems.

Fletcher and others (2001) inquired about the efficacy of some chemicals in their role as osmoprotectants, testing 12 chemicals with similar structure to betaine and proline, compounds that have already proved to confer osmoprotection to *Salmonella* (Csonka 1981). Results found that glycine betaine was the most powerful osmoprotectant followed by proline betaine, pipercolate betaine, dehydroproline betaine, and others. *Enterobacteriaceae* possess an assortment of adaptive and non-adaptive responses to high osmolarity environments, among them, there is the accumulation of K<sup>+</sup> ions, glutamate, trehalose, reduction in water content and cytoplasmic volume. Additionally, the induction of the *proU*, *prop* and *kdp* operons and approximately 20

other DNA fragments related to osmoregulation becomes apparent when stress is induced in the cell.

*Escherichia coli*, as a member of the *Enterobacteriaceae* family, may share some of the same stress response mechanisms with other genera of the same family, like *Salmonella*. The first response of *E. coli* to osmotic stress is the uptake of  $K^+$  ions, then, synthesis of glutamate takes place. As the time progresses,  $K^+$  and its counterions are removed by osmoprotectants, such as trehalose and glycine betaine. Synthesis or import of osmoprotectants into the cytoplasm is stimulated through the response of osmotically regulated genes. Bianchi and Baneyx (1999) studied the interrelation of  $\sigma$  regulons in the adaptation of *E. coli* to an osmotic aggressive environment. In *E. coli* the induction of the stress response mechanism is coordinated by several sigma factors ( $\sigma$ ). These factors, when activated, draw the RNA polymerase core enzyme E to the initiation region of the adaptation genes. The *rpoS* gene encodes  $\sigma_s$ , a sigma factor that oversees more than 50 genes, whose expression is triggered as a response to harsh environments, such as osmotic or acid stress. When *E. coli* cells were in the exponential growth phase and went through an osmotic shock, the translation of the *rpoS* gene was increased. The results of Bianchi and Baneyx (1999) study suggested a relationship between the  $\sigma^{32}$ ,  $\sigma^E$  and  $\sigma^S$  regulons to control the osmotic stress in the cell. Activation of the  $\sigma^{32}$  and  $\sigma^E$  regulon seems to be a support-stress response mechanism with the objective of aiding in the correct folding of the newly formed proteins. Augmenting the activity of  $\sigma^S$  appears to be the main strategy to achieve osmoadaptation of the cell. Similarly, Bang and others (2005) stated that the induction of a particular set of genes depended on the stress to

what *S. Typhimurium* was exposed. Extracytoplasmic, thermic or nutrient limitation stresses triggered the sigma factors  $\sigma^E$ ,  $\sigma^H$ ,  $\sigma^S$ , respectively. These sigma regulons are known to be indispensable for the battle against oxidative stresses. Gunasekera and others (2008) described the response of extracellular osmolarity and temperature on the expressed genes of *E. coli* K-12. The most important finding was that some genes in the SoxRS and OxyR oxidative-stress regulons were up-regulated by high osmolarity, high temperature, or a combination of both stresses.

Mattick and others (2000a) investigated the survival of *S. Enteritidis* PT4 and *S. Typhimurium* DT104 in broths with adjusted *A<sub>w</sub>* by means of NaCl, sucrose or glycerol addition. Inoculated broths were incubated for 5 months at 21 and 37 °C. This experiment was simultaneously performed on mutants *S. Enteritidis* PT4 and *S. Typhimurium* DT104 lacking the *rpoS* gene. This study confirmed the survival of *Salmonella* at low *A<sub>w</sub>* and its dependency on the type of solute. Additionally, the expression of the *rpoS* gene was required for a higher survival rate. Mattick and others (2000b) studied the heat resistance of three strains of *Salmonella* and one mutant lacking the *rpoS* gene. In their experiments, strains were habituated to an environment of a *A<sub>w</sub>* of 0.95, recreated by the addition of glucose-fructose, NaCl or glycerol. Cultures were exposed to a heat treatment at 54 °C. As observed by other authors, habituation at a *A<sub>w</sub>* of 0.95 elicited an increase in heat tolerance. The habituation period and type of solute were relevant in the heat tolerance increase. The D-value was increased four-fold when cells were conditioned in the glucose-fructose broth for 12 h. Additionally, Mattick and others (2000b) investigated the role of protein synthesis in the heat tolerance of

habituated cell. The  $A_w$  of nutrient broths was adjusted to 0.95 using glucose-fructose as solutes. The nutrient broths were then supplemented with protein-inhibitor-antimicrobials, rifampicin or chloramphenicol. The mechanism for the antimicrobial activity of rifampicin is by inhibiting the transcription process of the cell, whereas, chloramphenicol inhibits the translation process (Forbes and others 2002). The addition of rifampin or chloramphenicol had no effect on the heat tolerance of *Salmonella*, suggesting that heat tolerance is independent of the proteins produced at a  $A_w$  of 0.95, contradicting to what Fletcher and Csonka (1998) had published in regard to the production of chaperoins and proteases. However, the author stated that it is possible that when osmotic stress rises, the inhibition of protein synthesis by antibiotics is bypassed.

### **Almonds and other low moisture food as vehicles of *Salmonella***

#### *Paprika and paprika powder*

In 1993, Germany experienced a nationwide outbreak of salmonellosis. The transmission vehicle was paprika and paprika-powdered potato chips. Epidemiological investigations estimated that the extent of the outbreak was 1,000 cases, most of which were children below 14 years of age. The estimated infectious dose was established between 4 and 45 cells. Uncommonly, there were three *Salmonella* strains involved, *S. Saintpaul*, *S. Rubislaw* and *S. Javiana*. Clinical isolates were matched with paprika and paprika containing products (spice mixes and snacks) using pulsed field gel electrophoresis (PFGE) techniques (Lehmacher 1995).

### *Peanut butter and peanut paste*

Peanut butter was first implicated in a salmonellosis outbreak in the U.S. in 2006-2007. *Salmonella* Tennessee was identified as the etiologic agent, and the outbreak was linked to a specific brand of peanut butter. During this outbreak, more than 600 people were infected (CDC 2009). According to a press release by the manufacturing company, the mechanism of contamination was a leakage from the ceiling over finished product (ConAgra 2007). A second outbreak occurred in 2008-2009, implicating peanut butter and peanut paste manufactured by a single plant. *S. Typhimurium* was isolated from clinical samples, intact packages of food, and the processing plant environment (CDC 2009). The relationship between the clinical samples and the peanut butter and peanut paste isolates was confirmed through PFGE. As a result of this outbreak, a total of 116 people were hospitalized. It was estimated that the salmonellosis infection might have aggravated the health of 8 patients to fatal consequences. A remarkable characteristic of this outbreak was the recall of at least 431 food products involving 54 companies which used peanut butter or peanut paste as an ingredient, making this recall one of the largest in the U.S. As a result of the outbreak, the manufacturer of the peanut butter and peanut paste, Peanut Corporation of America, filed bankruptcy in 2009 (CDC 2009, PCA 2009).

### *Cocoa powder and chocolate*

Since the 1960's, outbreaks in the confectionary and chocolate industry have identified *Salmonella* as the etiological agent responsible of hundreds of illnesses (D'Aoust 1977). Between 1973-1974 an outbreak in Canada and the U.S. identified

chocolate candy as the vehicle, however, it has been suggested that the probable source of contamination was the cocoa beans (D'Aoust 1977). Similarly to other low  $A_w$  foods, the infectious dose was low, in the range of 20-90 cells per 100 g (D'Aoust 2000). A more recent outbreak incriminating chocolate products manufactured in Germany and distributed in Europe and Canada was investigated in 2001. The PFGE profiles of *S. Oranienburg* from human isolates were indistinguishable from the chocolate sample isolates. The infection dose was estimated from 1.1-2.8 cells/g (Werber 2005). In 2006, Cadbury chocolates were implicated in a *S. Montevideo* outbreak. There were 42 confirmed cases, and the population median was 4 years old. Clinical isolates had an indistinguishable PFGE profile compared to finished product, and samples from the manufacturer premises. According to the Health Protection Agency from the United Kingdom (UK), a leaking pipe was thought to be the origin of contamination. Cadbury was sued, plead guilty, and fined by the UK government for £1,000,000. The entire cost of the incident, including the additional costs of recall, and brand damage, was estimated to be 45.5 million Euros (HPA 2010).

### *Almonds*

Raw almonds were first implicated in a salmonellosis outbreak in 2001. The isolates were identified as phage type (PT) 30, and there were 157 confirmed cases in Canada and 11 in the U.S. (Isaacs and others 2005). Due to this outbreak, several tons of raw almonds were recalled, and Danyluk and others (2007) determined that the prevalence of *Salmonella* in the recalled product was 84%, with a level of 8.5 MPN/100

g. In 2004, there was a second outbreak implicating raw almonds. In this case, the isolates were identified as PT 9c (Danyluk and others 2007) and there were 47 confirmed cases between the U.S. and Canada (Harris and others 2009). In 2005, a cluster of 15 cases of *S. Enteritidis* infection was reported in Sweden. An epidemiological case-control study determined that raw almonds had an unmatched odds ratio of 45. However, the pathogen was never isolated from tested almonds. The PFGE pattern differed from the isolates responsible of the outbreak in the U.S. and Canada in previous years (Muller and others 2007). Although the source and mechanism for the contamination of almonds with *Salmonella* is still unknown, it was suggested that typical agricultural practices such as harvesting, drying and hulling-shelling may have allowed for the contamination of the nuts (CDC 2004).

#### *Pistachio recall*

In March of 2009, the FDA notified the CDC that pistachio and pistachio-containing samples from a single processor, Setton Pistachio of Terra Bella, Inc., were contaminated with *S. Montevideo*, *S. Newport* and *S. Senftenberg*. These pistachios were distributed to sell as whole-nut or as an ingredient for cakes, cookies, trail mixes, snack bars, ice cream, and others. Despite the inconclusive evidence to relate human illness with contaminated pistachios, the manufacturer announced a voluntary recall as precautionary measure. This was the first recall in the history of the pistachio industry in the U.S. (FDA 2010b).



## **Controlling *Salmonella* in almonds**

### *Good agricultural practices as a prevention strategy*

In 2009, the ABC published Good Agricultural Practices (GAP) guidelines to inform producers of the inherent risks of growing, harvesting and processing almonds. This set of suggestions stresses the importance of prevention to minimize the risk of contamination. Almonds are at risk of being contaminated with biological, physical and/or chemical hazards. The biological hazards in almonds are *Salmonella* and *E. coli* O157:H7, or any other Shiga toxin producing *E. coli* (STEC). Physical hazards include stones, glass and metal. Among chemical hazards there are pesticides, food allergens and aflatoxins (ABC 2009b).

The document lists 8 basic principles of GAPs, which are: documentation and traceability, employee training, fertilizer and soil amendment practices, water quality and source, field sanitation and worker hygiene, orchard floor management, pest control, harvest and delivery sanitation. In the documentation and traceability section growers are encouraged to document at least three events: orchard practices prior to harvest, establishment of lot numbers and records that enable to locate product one step back to the orchard and one step forward to the huller/sheller or customer. The employee training section highlights the importance of regular training and the corresponding documentation. Specific training in areas such as hand-washing techniques and facilities is recommended. The involvement and commitment of management towards the implementation of safety procedures needs to be communicated during the training sessions, this includes policies and reports in regard to illnesses and personal injury. The

fertilizer and soil amendment practices highlight the importance of maintaining records of production history, previous land use, and adjacent land use. This section also includes recommendations on soil testing for pathogen indicators when dairy or poultry operations, or high use of animal manure practices were performed at the orchard. Due to the extensive contact of almonds with the orchard soil during harvesting practices, it is crucial that the risk of pathogens presence on the ground is minimized. Therefore, the ABC does not advocate the use of manure, but the GAP document provides education on the proper handling, treating, storing and testing of manure. The water quality and source section explains the importance of learning about the primary and secondary sources of water, prevention of water contamination and disinfection procedures. Whether the water sources include wells, surface, or municipal district water systems, the ABC recommends to bimonthly test for total fecal coliforms and generic *E. coli*, to develop a baseline for use in a monitoring system. Water used in the dispersion of pesticide and foliar feed applications should come from a tested source. The document stresses the importance of revising water systems where back-flow is likely to occur. The orchard floor management fragment stresses the unavoidable contact of almonds with the orchard floor, and the potential contamination with *Salmonella*. Some of the recommendations include: the restraint of domestic and wildlife animals, identification of surrounding dairy production farms and their manure management program, development of a flood-disaster plan, monitoring of soil moisture, and avoidance water pooling by having a smooth, hole-free orchard floor. In the field sanitation and worker hygiene recommendations, the ABC emphasizes that providing well-maintained toilet

facilities to orchard employees are mandated by federal and state regulations. Additionally, proper and documented training on hand washing procedures, as well as good quality stations and supplies in the field, are necessary. The ABC advises the implementation of a pest control system. By implementing such a system, current and potential sources of contamination might be reduced. The system must contain specific procedures on the safe use of pesticides and the corresponding record keeping. In the harvest and delivery sanitation segment, the ABC highlights the importance of implementing a cleaning and sanitation program for machines, tools used at the time of harvest, transport and storage sites. This program must include records of the past use of the machinery, if it has been used in manure-using farms or not, and equipment design and maintenance schedule. In addition, almond hulls on the floor need to be kept as dry as possible to prevent mold and bacteria, such as *Salmonella*, growth. Stockpiling is a practice that if not properly monitored may lead to mold infestation. This is the reason for maintaining almonds in hulls at a moisture level below 6.5-7% and practice adequate fumigation. Moreover, verification of the huller/sheller sanitary and pest control practices are paramount in ABC's commitment to maintain the best quality product.

*Interventions for pathogen control and current regulatory requirements for almonds*

After the two outbreaks where almonds were found implicated as the transmission vehicle, the ABC felt the need to reinforce their commitment to the consumer in providing the safest and highest quality product. Therefore, in 2006 the ABC board of directors approved an "Action Plan" with a goal to ensure that all almonds

from California are treated to reduce potential pathogen contamination (ABC 2008). Danyluk and others (2006) performed a Monte Carlo risk analysis simulation finding that a treatment of raw almonds that achieved a 5-log reduction of *Salmonella* would drop the risk of one or more cases of salmonellosis in the U.S. from a 78% to 1%. The Final Rule for the Mandatory Pasteurization of California Almonds was published in the Federal Register as of March 30, 2007 and mentions that almonds must be subjected to a treatment process or processes that achieve in total a minimum 4-log reduction of *Salmonella* bacteria. The effective date for implementation of the rule was September 1, 2007 (ABC 2008).

According to Salin and others (2006) almond processors treating their product by blanching or oil roasting could find less expensive technologies to implement a pathogen control intervention. Several technologies have been recognized to have the ability to comply with the newly approved regulation. Up to 2009, the ABC (2010d) has confirmed the validity of the following processes: water blanching, steam processing, oil roasting, dry roasting, and fumigation with propylene oxide (PPO). Other technologies that have proved to reduce more than 4 log CFU/g of *Salmonella* are: electron beam irradiation (Prakash and others 2010), infrared heat (Brandl and others 2008), and high hydrostatic pressure (Willford and others 2008).

### *Heat treatments*

The industrial application of heat with the aim of food preservation has been scrutinized since the 1800's (Jay 2005). The mechanism of action of thermal treatments

involves the denaturation of proteins, which can be tissue enzymes, or in microorganisms, metabolic enzymes and transport proteins (Ramesh 2007).

Pasteurization is a moderate heat treatment below 100 °C, with the purpose of destroying pathogens and shelf-life extension (Fellows 2002). The National Advisory Committee on Microbiological Criteria for Foods (NACMCF 2004) defined pasteurization as “Any process, treatment, or combination thereof, that is applied to food to reduce the most resistant microorganism(s) of public health significance to a level that is not likely to present a public health risk under normal conditions of distribution and storage”.

Thermal food processing is based on conventional concepts that have been established for more than a century (Knorr and others 2007). The concept of D-value relies on the premise that the rate of a population destruction is a first-order reaction, meaning that the same number of microorganisms will be destroyed in a given time interval despite the initial number. This theory is graphically expressed in a death rate curve, where the time required to destroy 90%, or 1 log of the initial population is referred to as the D-value, or the decimal reduction time, when exposed to a constant temperature. D-values are calculated as the reciprocal of the slope of the linear regression equation of a death rate curve. D-values depend on the type of matrix, microorganism and temperature settings (Berk 2009). Therefore, the collection of data for specific product-pathogen-process combinations is an ongoing process.

### *Blanching*

This is one of the processes approved by the FDA for the pasteurization of almonds (ABC 2008). This process was first conceived to aid in the removal of almond skins in the production of skinless almonds, used as ingredients for confectionery, energy bars, bakery, desserts, and other products (ABC 2010a). Blanching comprises three unit operations, being scalding, skin removal and drying. In the almond industry, scalding is usually achieved as a continuous process, carried out in an insulated metal pipe chamber with inlet and outlet sections. The chamber is filled with hot water that serves as a heat transfer medium, and carries the product through the circular chamber. The length of the chamber and the speed of the water dictate the residence time of the kernel in the chamber (Fellows 2002).

Once the kernels have reached the exit window of the chamber, they are passed through a series of rubber rollers to remove the loosened skins and aspirators remove the skins. Skinless almonds are rinsed with water to remove any remaining skins and finally transported to a continuous dryer. Thermocouples around the chamber and a speed dial component monitor the correct functioning of the scalding (ABC 2010d, Fellows 2002). To ensure that the blanching process complies with the mandatory pasteurization of almonds criterion, appropriate parameters were investigated by researchers at the University of California at Davis. Uesugi and Harris (2005) determined that a blanching process of 2 min or more with water at 88 °C or more, provided the required lethality. The FDA has issued a letter of determination recognizing these conditions as an appropriate pasteurization process (ABC 2010d).

### *Oil roasting*

This method is widely used in the almond industry originally implemented with the purpose of favorably altering the flavor and texture of raw almonds. In some cases, this process is paired with salting and flavoring. Commercially, almonds are discharged into a hot oil tank assembled with a conveyor belt that transports the almonds throughout the entire length of the tank. Critical parameters to monitor are the speed of the belt, and the volume and temperature of the oil and the incoming almonds. Common time-temperature conditions to achieve a good crispy roasted almond are 3-15 min at 137-176 °C (ABC 2010d). The ABC sponsored validation studies to establish the minimum parameters to comply with the newly passed mandatory pasteurization of almonds because scientific literature evaluating the efficacy of oil roasting as an intervention to control *Salmonella*, is limited. According to Du and Harris (2005), a minimum exposure time of 2 min in oil at 127 °C provides a 5-log reduction of *Salmonella*. In 2006, the authors submitted a report to the FDA, which resulted in a letter of determination endorsing the *Salmonella* control capabilities of this technology.

### *Other thermal technologies*

The use of steam to control *Salmonella* is one of the pasteurization technologies approved by the ABC. Lee and others (2006) investigated the application of steam onto Nonpareil and Mission almond varieties, when artificially inoculated with three strains of *S. Enteritidis*; 43553, ME-13, and ME-14. Almonds were treated for up to 65 s with the resulting steam from a 93 °C water steam pot. Calculated D-values for Nonpareil and

Mission varieties were significantly different, 12.22 and 16.13 s, respectively. Chang and others (2010) evaluated the use of steam pasteurization to control *S. Enteritidis* PT 30 inoculated on raw Nonpareil almonds. Almond surfaces were first treated with steam at 95 °C for 5 to 65 s. Then, to cool down the almonds, compressed air at 22 °C was injected for 5 s. Finally, almonds were dried with a fan at 22 °C for 10 min. Twenty five s of the steam treatment was found to be sufficient to achieve a 5 log reduction of *S. Enteritidis*. Brandl and others (2008) examined the effect of infrared (IR) heat as a decontamination strategy for raw almonds. Nonpareil raw almonds were inoculated with *S. Enteritidis* PT 30 and treated with a double-sided catalytic IR heating system of 5.45 kW/m<sup>2</sup>. Almonds were IR heated up to 45 s, followed by a cooling down period of 15 min at room temperature. Results showed a reduction of 1.5 log CFU/g in the *Salmonella* population. In a second experiment, the authors tested the abilities of the technology when combining it with a pre-wetting step. When the two-step cycle was repeated 3 consecutive times, it resulted in a reduction of 3.6 log CFU/g of *S. Enteritidis* PT 30. In a third test, the surface temperature of almonds was elevated to 109 °C by IR heating, followed by a 60 min holding-period at 80 °C. This was the most effective treatment at controlling *S. Enteritidis* PT 30, reaching a 7.5 log CFU/g reduction. Jeong and others (2009) assessed the efficacy of moist-air convection heating as a strategy to control *S. Enteritidis* PT 30. The parameters evaluated included temperature (121 to 232 °C), moisture by volume (% Mv) (5 to 90%), and exposure time (5 to 1,800 s). In general, temperature variations within the same % Mv produced minimal changes in *Salmonella* reductions. However, variations in % Mv, while maintaining the temperature



constant, contributed to an increase in *Salmonella* reductions. Finally, heating time was directly related to *Salmonella* reductions. Several treatment combinations were able to reduce more than 5 logs of CFU/g of *Salmonella*. Bari and others (2010) studied the effectiveness of combining superheated steam (SHS) and gas catalytic IR heat interventions to control several strains of *S. Enteritidis* inoculated onto raw Nonpareil almonds. Inoculated almonds were sprayed with steam at 115 °C for 20 to 120 s, held for 1 min at room temperature, and dried for 70 s using an IR heater. Reductions of approximately 5.7 log CFU/g were achieved after applying the steam treatment for 70 s. Yang and others (2009) determined the effect of three roasting treatments on Nonpareil almonds inoculated with *Enterococcus faecium* NRRL B-2354, a surrogate for *S. Enteritidis* PT 30. The greater effect was observed when inoculated almonds were heated to 150 °C using an IR heater, and the temperature was held for 5 min using a hot air roaster. These conditions produced a reduction of 7.0 log CFU/g of *E. faecium*. These settings allowed for a medium roast almond. Bari and others (2009) evaluated the effectiveness of sanitizers, dry heat, hot water and IR heating alone and in combination, with the purpose of reducing several strains of *S. Enteritidis* inoculated onto Nonpareil almonds. Sanitizer dips, such as strong and mild electrolyzed water, ozonated water, and distilled water, were tested for 10 s. The strong electrolyzed water treatment obtained the highest reduction, achieving a 0.6 log CFU/g reduction. Treating the inoculated almonds with IR heating alone, achieved a reduction of 1.2 CFU/g. Combining the sanitizer dip with IR heating for 70 s, reduced up to 3 log CFU/g. Subjecting the inoculated almonds to a dry heat treatment at 60 °C for 4 d, followed by IR heating for 70 s accomplished a 4

log CFU/g reduction. The best treatment, 4.7 CFU/g reduction, was found to be blanching at 85 °C for 40 s, followed by IR heating for 70 s.

### *Non-thermal technologies*

Propylene oxide (PPO) has been approved since 1958 for use as a pesticide on foods by the FDA. Currently, foods such as cocoa, spices and nuts are treated with PPO (Navarro and others 2004). In 2004, the FDA extended a letter of determination confirming the efficacy of PPO as a pasteurization treatment in raw almonds (ABC 2010d). The PPO treatment consists of a pre-warming step between 43 and 49 °C for 48 to 96 h. Then, bins filled with approximately 900 kg of almonds are loaded into fumigation chambers. Then, vacuum (9.8 kPa) is pulled and 0.5 kg/m<sup>3</sup> of PPO is injected for 4 h. Finally, off-gassing takes place at a warehouse between 38 to 43 °C for 2 d, before tempering between 15 and 18 °C for an additional 3 d. Danyluk and others (2005) reported that *S. Enteritidis* PT 30 on almonds was reduced by 2.5 log CFU/g immediately after the PPO treatment was applied. Furthermore, a reduction of 4.4 to 7.2 log CFU/g was achieved after the off-gassing and tempering period, respectively. Pao and others (2006) evaluated the efficacy of organic acid sprays to eliminate *Salmonella* from raw almonds. Shelled almonds inoculated with *S. Enteritidis*, *S. Montevideo*, *S. Newport* and *S. Typhimurium* were sprayed with solutions of acetic acid, citric acid, acidified sodium chlorite, peroxyacetic acid and a mixture of hydrochloric, phosphoric and citric acid at various concentrations and exposure times. Treatments were applied in the following manner: for each solution, a single layer of 25 g of inoculated almonds

was placed on a weight boat. Then, solutions were sprayed, and almonds were shaken to ensure that the entire almond surface was wet. A reduction of 2.2 and CFU/g of *Salmonella* was achieved when 1.6 ml of 10% citric acid were sprayed. The largest reduction (4.0 CFU/g) was obtained when 3 applications (1.6 ml each) of 15% acetic acid were sprayed and a holding time of 40 s between each application was allowed. Reductions were directly correlated to the volume sprayed. The larger the volume, the greater the reduction. These authors (Pao and others 2006) also evaluated the delayed effect of organic acids after storing the treated almonds for up to 7 d. The greatest reductions (3.7 CFU/g) were obtained after 3 spraying cycles with 10% citric acid, followed by rinsing with water, and stored for 3 d. The effect of high hydrostatic pressure (HHP) for the means of reducing *S. Enteritidis* from almonds was investigated by Goodridge and others (2006). A pure culture of *S. Enteritidis* pressurized at 60,000 psi at 25 °C for 5 min was reduced more than 7.5 log CFU/ml. However, when *S. Enteritidis* was inoculated onto almond surfaces and treated under the same process conditions, the reduction was 0.8 log CFU/g. Greater reductions were achieved (1.3 log CFU/g) using HHP as a discontinuous process where inoculated almonds were subjected to 6 cycles of 60,000 psi, 50 °C, for 20 s, with a 30 s-rest-period in between each cycle. The efficacy of HHP was increased even further (*S. Enteritidis* reduction of 2.7 log CFU/g) when the treatment was applied onto inoculated almonds previously submerged in water. Prakash and others (2010) investigated the effect of electron beam irradiation as a pathogen intervention to control *S. Enteritidis* PT 30, *S. Anatum*, *S. Hartford* and a cocktail of *S. Anatum*, *S. Infantis*, *S. Stanley*, and *S. Newport*. Inoculated Nonpareil

almonds were irradiated at 1, 1.5, 2, 2.5, and 3 kGy. D-values for all the strains ranged between 1.06 and 1.25 kGy. Deng and others (2007) evaluated the effect of nonthermal plasma (NTP) in the inactivation of *E. coli* as a surrogate of *Salmonella*, according to the authors. Inoculated almonds were treated with NTP for a combination of different time intervals, voltages and frequencies. A 5-log reduction on the inoculated *E. coli* was achieved after a 30 s treatment with 30 kV and 2000 Hz process.

The combination of more than one hurdle to reduce *S. Enteritidis* PT 30 and *S. Enteritidis* PT 9c populations, was considered by Willford and others (2008). Application of HHP, drying, and heating were the three hurdles. The entire treatment consisted of the application of HHP at 414 MPa, 25 °C, for 6 min in almonds submerged in water, followed by drying and heating at 115 °C for 25 min. Synergistic effects were observed in this treatment combination capable of reducing at least 6.6 log CFU/g of *S. Enteritidis*.

### **Electron beam irradiation as an intervention**

Most of the FDA-approved almond pasteurization technologies are based in the application of heat (ABC 2008). Moreover, other *Salmonella* serotypes that are known to be more resistant to heat treatments, such as *S. Senftenberg* 775 W (Henry and others 1969), are at risk of surviving current heat treatment methods. Hence, research to identify alternative methods to pasteurize almonds is a current priority. Electron beam irradiation is a technology that encompasses more than 50 years of research. It is widely recognized that radiation treatments are beneficial to foodstuff including, shelf-life

extension, sprouting prevention, pest and pathogen control (Morehouse and Komolprasert 2004).

Food irradiation has proven to be safe enough to be granted approval from the FDA to be used on spices, meat, poultry, oysters, seeds for sprouting, and lately iceberg lettuce and spinach (FDA 2009). Electron beam irradiation has demonstrated to be an effective treatment for reducing foodborne pathogens such as *Salmonella*, *E. coli* O157:H7, *Listeria*, *Campylobacter*, *Yersinia* among others (Hayes and others 1995).

#### *Mode of action*

When accelerated electrons interact with matter, in this case foods, they encounter collisions with other electrons and nuclei, preceding energy losses and trajectory inflexions. Energized electrons used in food irradiation are regulated to carry less than 10 MeV (Morehouse and Komolprasert 2004). At these energy levels, the relativistic mass of an energetic electron is considerably larger than the mass of an atomic electron, but significantly smaller than an atomic nucleus. Due to these mass differences, the result of an energized-atomic electron collision, or inelastic collision, will be the transfer of considerable amounts of energy. In the case of a low energy transfer, the result will be the excitation of the atom. However, if this energy transfer is large enough, it will displace the electron from the atomic orbit. These released electrons are the so called secondary electrons. Secondary electrons will continue their path until a further collision decreases their energy or scatters them (Miller 2005, Turner 2007). Alternatively, an elastic collision is between an atomic nucleus and an energized

electron, which renders the energized electron with a scatter trajectory. Additionally, considerable energy loss leads to the emission of photons, a process called Bremsstrahlung. These collisions are a process that continues until the electrons reach a state of rest (Miller 2005, Turner 2007).

Cell death is mainly achieved by the extensive DNA damage exerted by the radiation source. This damage to the genetic material renders the cell incapable of exercising most of the cell functions, including reproduction. There are two main mechanisms of microbial inactivation by irradiation: direct, from the irradiation source, or indirect, from the oxidative damage caused by the reactive oxygen species generated as products of water radiolysis (Ahu and Lee 2006).

The direct contact of an electron onto the bacterial DNA helix can cause the rupture of one or both DNA strands. A single strand breach may not be lethal to the cell, but it may lead to mutations. However, if there are multiple single-strand lesions, the cell may not be cable of repair, leading to permanent inactivation of the cell. Conversely, a double strand lesion detaches the helix in two parts, leading in most cases to the cell's destruction. Nevertheless, the probabilities of receiving a double strand hit by the irradiation source are very limited (Dickson 2001).

Most food materials contain large amounts of water, which is also exposed to the effects of the irradiation source. The final products of water breakdown are the hydroxyl radicals,  $\text{-OH}^\cdot$ , and  $\text{H}_2\text{O}_2$ . These highly reactive compounds interact with the bacterial DNA, reaching the hydrogen bonds that link the two helix strands, and the phosphodiester bonds that link nucleotides to each other. These aggressive interactions

can lead to single and double strand ruptures with the same consequences as explained previously (Dickson 2001).

### *Challenges to overcome in the irradiation of almonds*

The two major challenges food irradiation must overcome are quality damage to the food, and consumer concerns. Quality damage can be minimized by controlling the applied dose (Patterson and Loaharanu 2000). Numerous studies have found, in many cases, the quality loss is not significantly different when compared to untreated product. Moreover, shelf-life extension is a common benefit when irradiating foods (Blank and Cumming 2001, Arvanitoyannis and others 2009, FDA 2009). As other technologies have previously experienced, the lack of information at the consumer level has influenced the decision to buy irradiated food. In a survey of 484 consumers, Nayga and others (2005) found that 50% of buyers at a grocery store were willing to purchase irradiated food. After informing the consumers of the nature and the benefits of food irradiation, the percentage of consumers willing to buy increased to 89%.

The possibility of a negative effect of irradiation treatments on the quality of the almonds is a concern that needs to be taken into consideration. Due to the high fat content of almonds (approximately 50%), lipid oxidation is the main challenge to overcome when applying irradiation (Fenemma 2000, USDA 2010). Several scientific papers have evaluated the sensory attributes of almonds and almond products. The majority of the studies have used a descriptive sensory technique with panel members, or a limited consumer group.

Narvaiz and others (1992), studied the lipid behavior of gamma irradiated almonds by chemical and sensory tests. They found that at a maximum dose of 2 kGy free fatty acid values remained unchanged in almonds stored at 2 °C over 160 d, whereas peroxide values increased significantly over time. In addition, sensory evaluation performed by five panel members concluded that the sensory attributes of irradiated almonds that were stored for 180 d at 2 °C were not significantly different from the non-irradiated controls. Sánchez-Bel and others (2005) evaluated the oil quality of almonds treated by electron beam irradiation at 3, 7 and 10 kGy. This research showed that the peroxide values of almonds irradiated at 7 kGy or less, and stored at 20 °C for 5 months were not significantly different from those of non-irradiated controls. In the same study, a trained panel of 5 members found no significant differences in rancidity and overall quality of untreated almonds and almonds-irradiated at 3, 7 or 10 kGy. Zacheo and others (2000) determined the degree of rancidity in 4 varieties of Italian almonds. A semi-trained panel consisting of ten members scored rancidity using a 4-point scale, with 1 as the highest and 4 the lowest. Each variety had 3 samples, fresh, frozen for 24 and 36 months. Panel members scored higher rancidity in the 36 months samples compared to the 24 and the control, for all the varieties. Senesi and others (1996) used a semi-trained panel of 10 members to evaluate the color, taste and acceptance of almonds after 4, 8 and 12 months of storage at 2 °C. Panelists scored on a 9-point scale. After 4, 8 and 12 months of storage at 2 °C the mean taste acceptance was 6.21, 6.7 and 5.7 respectively.

The determination of D-values of *S. Enteritidis* PT 30 and *S. Senftenberg* to e-beam is the first step in considering the use of this technology in the treatment of



almonds. Alongside, commercial packaging engineering, chemical and sensory quality evaluations will construct a robust technical assessment of e-beam irradiation for almonds.

## MATERIALS AND METHODS

### General

#### *Almond acquisition and storage*

Blanched, and roasted Nonpareil almonds were purchased from Treehouse California Almonds, LLC. (Delano, Calif.) and raw Nonpareil almonds were donated by the same company. Almonds were shipped to the Food Sensory Laboratory at Texas A&M University (TAMU, College Station, Tex.) where they were stored in a walk-in cooler at 4-7 °C until experiments were conducted. On the same day that almonds were received from the supplier, the  $A_w$  was recorded to determine a  $A_w$  baseline level.  $A_w$  measurement was achieved by retrieving 3 almond samples weighing  $20 \pm 1$  g from the original package and individually grinding them for 1 min using a food processor (Black and Decker, Baltimore, Md.). The almond powder was immediately transferred to disposable 15-mL sample cups (Decagon Devices, Inc., Pullman, Wash.) to fill one-half of their capacity, and then covered with airtight lids to prevent any gain or loss of moisture from the environment. One at a time, each cup was placed in the water activity meter (AquaLab Series 3, Decagon Devices, Inc.) and the  $A_w$  was displayed on the digital screen at the end of the equilibration period.

#### *Bacterial cultures*

*Salmonella enterica* serovar Enteritidis PT 30 ATCC 1045 was purchased from the American Type Culture Collection (ATCC, Manassas, Va.) and *Salmonella enterica*

serovar Senftenberg ATCC 43845 was obtained from the bacterial culture collection of the Food Microbiology Laboratory at TAMU. Individual stock cultures were maintained in cryopellets (Key Products, Round Rock, Tex.) at  $-80\text{ }^{\circ}\text{C}$ . Each strain was activated by transferring one cryopellet into tryptic soy broth (TSB, Difco, Sparks, Md.) followed by incubation at  $35\text{ }^{\circ}\text{C}$  for 24 h. Then, both strains were streaked on tryptic soy agar slants (TSA, Difco) and maintained at  $25\text{ }^{\circ}\text{C}$  until needed.

To obtain traceable derivatives of the *Salmonella* strains, natural mutants of *S. Enteritidis* PT 30 and *S. Senftenberg* that expressed rifampicin resistance (rif<sup>+</sup>) were selected by a modification of the method proposed by Kaspar and Tamplin (1993). In this modified method, both strains were individually transferred into 10 mL of TSB and incubated for 24 h at  $35\text{ }^{\circ}\text{C}$ . A 500- $\mu\text{L}$  aliquot was spread onto TSA supplemented with 100  $\mu\text{g}/\text{mL}$  of rifampicin (Sigma-Aldrich, St. Louis, Mo.) (TSA+rif) and incubated for 24 h at  $35\text{ }^{\circ}\text{C}$ . Of the few colonies that were able to grow, which were resistant to rifampicin, one colony was recovered and restreaked for isolation onto TSA+rif, and incubated for 24 h at  $35\text{ }^{\circ}\text{C}$ . Finally, a single colony was streaked onto a TSA slant and used as a working culture.

To confirm the identity of the rif<sup>+</sup> mutants, *S. Enteritidis* rif<sup>+</sup> and *S. Senftenberg* rif<sup>+</sup> were streaked onto TSA plates and incubated at 24 h at  $35\text{ }^{\circ}\text{C}$ . Then, a single isolated colony from each culture was scraped from the agar surface with a sterile cotton swab and the cells were suspended into approx. 3 ml of saline (0.45% sodium chloride) (Allegiance, Jackson, MI). An API 20 E (bioMérieux SA, Marcy l'Etoile, France) strip was inoculated with the previously prepared bacterial suspension. Instructions from the

manufacturer were followed to read individual biochemical tests and identify the isolate. Rif<sup>+</sup> strains were tested to ensure that the induced resistance produced no adverse effects on their biochemical, and growth characteristics, as well as their irradiation and heat resistance. Details of the comparison experiments can be found in the Preliminary experiments section.

#### *Selective and differential media development*

To aid in the identification between *S. Enteritidis* PT 30 rif<sup>+</sup> and *S. Senftenberg* rif<sup>+</sup> inoculated in the same sample, TSA+Rif was supplemented with 0.2 g of FeSO<sub>4</sub> and 0.3 g of Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub> to form modified TSA+rif (MTSAR). The reasoning behind the addition of these chemicals relies on the biochemical characteristics that each of these serovars express. *S. Enteritidis* PT 30 ATCC 1045 carries membrane-bound thiosulfate reductases encoded in the *phs* operon. These enzymes are able to reduce sodium thiosulfate (Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub>) to hydrogen sulfide (H<sub>2</sub>S) (Price-Carter and others 2001), which then, is combined with the FeSO<sub>4</sub> from the medium to render ferrous sulfide (FeS), a black precipitate that imparts an indistinguishable black color to the colonies. In contrast, *S. Senftenberg* ATCC 43845 does not have the ability to reduce thiosulfate (S<sub>2</sub>O<sub>3</sub><sup>=</sup>) into H<sub>2</sub>S. Therefore, colonies of *S. Enteritidis* PT 30 rif<sup>+</sup> are black in color, while *S. Senftenberg* rif<sup>+</sup> colonies are white when plated onto MTSAR.

## **Preliminary studies**

### *Growth characteristics*

Growth from TSA slants of *S. Enteritidis* PT 30 ATCC 1045, *S. Senftenberg* ATCC 43845, and their rifampicin derivatives, was individually subcultured in 10-mL of TSB. Inoculum for each strain was individually prepared by mixing 1 mL of the 24-h culture with 99 mL of peptone water (PW). After gentle agitation, 1 mL of the suspension was transferred to a second bottle of 99 mL of PW. This step was repeated twice. For each strain, a set of 30 TSB tubes, pre-warmed at 35 °C, were spiked with 1 ml of the inoculum. After inoculation, TSB tubes were gently shaken and incubated in a water bath shaker (Classic C76, New Brunswick Scientific, Edison, N.J.) at 35 °C and 250 rpm. A set of 3 tubes per strain was retrieved at 2 h intervals for up to 12 h, and submerged in ice-water. One-and-a-half mL of each replicate was individually transferred to an optical cuvette (Brand Tech Scientific, Essex, Conn.). A cuvette with 1.5 mL of uninoculated TSB was used as blank. Cuvettes containing the samples were loaded into a spectrophotometer (Biomate 3, Thermo Electron Corp., Madison, Wis.) carousel, and the optical density (O.D.) was measured at a 600 nm. O.D. is a dimensionless measure of turbidity, which represents the amount of microbial cells present in a broth. Therefore, following the increase of O.D. in a culture constitutes a measure of bacterial growth. This experiment was replicated in two different days. Kinetic growth parameters were obtained by plotting and fitting O.D. over time to a Baranyi regression equation using MicroFit v1.0 (Institute of Food Research, Norwich,

UK). Comparison of the growth parameters of ATCC strains and their rif<sup>+</sup> counterparts were performed by analysis of variance (ANOVA).

#### *Thermal resistance of rifampicin resistant Salmonella strains*

Comparison of the resistance of rif<sup>+</sup> *Salmonella* strains and their non-resistant counterparts at the blanching temperature (88 °C) was first attempted by the capillary method described in Cabrera Diaz (2007). The purpose of using these capillaries is that when they are placed in a water bath the heat transfer to the culture is almost immediate. In this capillary method, a 50 µL-aliquot is injected into a glass capillary of 1.0 mm of internal diameter, which is then heat sealed on both endings. For the current study, parent and rif<sup>+</sup> strains were individually filled into capillaries before they were heat sealed and submerged into a water bath at 88 °C for 5, 5 s intervals, from 0 s to 20 s. Capillaries were removed from the water bath after the corresponding exposure time, and immediately submerged into an ice-water bath. This experiment was performed with three repetitions. The rapid die-off rate of *Salmonella* cells suspended in an aqueous solution treated at 88 °C restricted the use of this method (> 8.1 log CFU/mL /s). As an alternative, and with the ultimate objective of using rif<sup>+</sup> *Salmonella* strains in almond challenging studies, *Salmonella* strains were inoculated onto almond surfaces. In brief, each parent and rif<sup>+</sup> strain was cultured in TSB, followed by a centrifuge wash and resuspension into phosphate-buffered saline (PBS, Calibrochem, EMD Biosciences Inc., La Jolla, Calif.). A standardized amount of almonds was individually combined with the bacterial inoculum of parent and rif<sup>+</sup> strains, and mixed by hand shaking. The almonds

were allowed to dry and to recover their original  $A_w$  inside an incubator. Then, heat treatments were conducted at 88 °C, with exposure times of 0, and 60 s. Details regarding the inoculation and blanching procedures can be found in the inoculation and blanching sections of this document. For each *Salmonella* strain, there were three repetitions per exposure time. The experiment was replicated in 3 different d. The counts of surviving bacteria were transformed to log CFU/g and fitted to a linear regression equation using SPSS Inc. (Statistical Package for the Social Sciences, IBM, Somers, N.Y.). Reductions (log CFU/g) between rif<sup>+</sup> and non-resistant *Salmonella* strains were compared using the ANOVA procedure of SPSS.

#### *Radiation resistance of rifampicin resistant Salmonella strains*

This procedure is a modification of the one described by Rodriguez and others (2006). The main modification to the aforementioned protocol was the use of TSA as a support matrix for rif<sup>+</sup> and non-resistant *Salmonella* strains. To ensure a uniform depth in every TSA plate, 20 ml of melted TSA was aseptically dispensed in disposable petri dishes. Agar plugs of 5 cm<sup>2</sup> were aseptically cut out using a sterile round 1” metallic borer. Plugs were placed in empty disposable petri dishes for further inoculation. The inoculum was prepared by subculturing each strain in 10 mL of TSB and incubating for 18-24 h at 35 °C. Then, each culture was transferred to conical centrifuge tubes and the cells were washed by centrifugation at 1,620 × g for 15 min. The pellet was resuspended in 10 mL of (PBS). Agar plugs were inoculated with 0.1-mL aliquots of bacterial suspension. For each strain, there were four sets of three plugs each. Agar plugs were

left to air-dry for 1 h inside a laminar flow hood (Model 250, Contamination Control Inc., Kulpsville, Pa.) Each set of three agar plugs were aseptically transferred to sterile stomacher bags. Vacuum was pulled in each bag before heat sealed using an industrial vacuum sealer (Model X180, Koch Inc., Kansas City, Mo.). Each bag was double bagged in another stomacher bag and heat sealed as well. Samples were transported to the National Center for Electron Beam Food Research (NCEBFR) at TAMU in an insulated cooler with refrigerant packs. Inoculated agar plugs were randomly assigned to irradiation treatments including, 0, and 1.4 kGy. After irradiation, samples were brought back to the Food Microbiology Laboratory. Then, each agar plug was aseptically transferred to a stomacher bag to where 99 mL of PW were added. After pummeling the sample for 1 min, serial dilutions in PW were prepared and plated onto TSA. Plates were incubated at 35 °C for 24 h before enumeration. Plate counts were transformed to log CFU/mL and fitted to a linear regression equation. Reductions (log CFU/g) between rif<sup>+</sup> and non-resistant *Salmonella* strains were compared using the ANOVA procedure of SPSS.

#### *Restoration of almond Aw after storage and inoculation*

The National Advisory Committee on Microbiological Criteria for Foods (NACMCF) (2010) had stressed the importance of maintaining the intrinsic characteristics of the food matrix when adding an inoculum during a challenge study. Recommendations to maintain the Aw of the food include, minimizing the inoculum volume, and concentrating the inoculum by centrifugation or by growth in solid media.



In the present study, these three recommendations were followed, however, deviations from the original Aw in the almonds triggered the search for a novel protocol to restore the Aw in inoculated almonds. Most challenge studies investigating the feasibility of interventions to reduce populations of *Salmonella* in almonds have overlooked the restoration of the Aw of the inoculated almonds, leaving their scientific conclusions questionable (Prakash and others 2010, Chang and others 2010, Bari and others 2009, Bari and others 2010, Pao and others 2006, Jeong and others 2009, Danyluk and others 2005, Willford and others 2008, Lee and others 2006, Deng and others 2007, Brandl and others 2008). Goodridge and others (2006), measured the Aw of inoculated almonds after 1, 2, 3 h of drying, and storage of 8 and 24 h. Unfortunately, the authors omitted the drying and storage conditions and only reported that the Aw was 0.15, after 24 h of storage.

Raw almonds were stored in a walk-in cooler at the Food Sensory Laboratory at TAMU until further needed. When almonds were retrieved from the walk-in cooler, the Aw was measured. Relative humidity conditions during the cold storage increased the Aw of the almonds. Therefore, before almonds were used in this study they were dried until their original Aw (0.60) was restored. Drying was performed as follows: 420 g of almonds were placed into a 45 x 45 cm metal wire rack and placed in an incubator (Gallenkamp/Sanyo, San Diego, Calif.) at 35 °C for 12 h, after which almonds' Aw was restored to the baseline level (0.60).

To restore the Aw of the almonds after inoculation, inoculation was simulated by placing a 400-g sample of almonds (Aw= 0.60) inside a polyethylene bag (40 x 36 cm)

(Pactiv Corporation, Lake Forest, Ill.) to which 25 mL of distilled water were added. Almonds and water were mixed by hand-shaking the bag for 2 min. A 20-g subsample of wet almonds was immediately recovered for Aw measurement. In order to restore the Aw of the almonds after the inoculation procedure, a drying operation was necessary. Wet almonds were placed onto 4 sheets of absorbent paper extended inside a metal rack and allowed to dry inside an incubator (Gallenkamp/Sanyo) for 12 h at 35 °C. After the drying operation, the Aw of simulated inoculated almonds was restored to the baseline level. This experiment was performed on three separate days in duplicate.

Since the Aw meter is located in a Biological Laboratory Safety Level 1 setting, simulated inoculation using distilled water instead of a true inoculum was conducted to comply with Biological Laboratory Safety Level 2 (BL2) requirements. Performing this experiment allowed for the design and establishment of a protocol to restore the Aw to baseline levels in inoculated almonds. This ensured that the pathogen of interest was challenged at the most realistic conditions possible.

### **Decimal reduction dose and time of *S. Enteritidis* rif<sup>+</sup>, and *S. Senftenberg* rif<sup>+</sup> on inoculated almonds using electron beam irradiation, blanching and, oil roasting**

#### *Inoculum preparation*

This procedure is a modification of the method described by Danyluk and others (2005). To activate the microorganisms, growth from each *Salmonella* strain was transferred from the TSA working slant into 10 ml of TSB and incubated at 35 °C for 24 h. Subsequently, 2 mL of the culture broth were transferred to a cell culture bottle

(Becton Dickinson Labware, Franklin Lakes, N.J.) with a TSA surface of 75 cm<sup>2</sup>. The inoculum was spread throughout the TSA surface by aseptically adding sterile glass beads and rotating the beads over the entire agar surface. Four cell culture bottles, for a total of 200 cm<sup>2</sup>, per strain were inoculated and incubated at 35 °C for 24 h to obtain a bacterial lawn. Growth from each cell culture bottle was harvested by adding 10 mL of PBS to each bottle and collected by swirling the glass beads left from the inoculation step and transferred with a pipette to sterile conical centrifuge tubes. At this point, both *Salmonella* serovars were combined to make a bacterial cocktail. This suspension was washed by centrifugation at 1792 x g for 15 min in a centrifuge (Jouan B4i, Thermo Electron Corp., Madison, Wis.) and the resulting pellet was resuspended in 25 mL of PBS. Serial 10-fold dilutions of this inoculum were prepared in sterile 0.1% PW, spread plated onto TSA and MTSAR and incubated at 35°C for 24 h before colony enumeration.

#### *Bacterial inoculation and Aw restoration*

Raw Nonpareil almonds were inoculated with the previously prepared inoculum. The same time and temperature conditions that were defined in the Aw restoration preliminary experiment were now used to inoculate raw Nonpareil almonds with a cocktail of *Salmonella* strains. To further assure the accuracy of the Aw measurement in the inoculated almonds a non-inoculated set was equally treated in parallel. Aw measurements were only performed for the non-inoculated set (S1), which was used as a non-inoculated control to determine the Aw of the almonds throughout the inoculation

process. The other set (S2) was inoculated with the bacterial cocktail prepared as described in the previous section. Two sets of 420 g each of raw Nonpareil almonds were placed into 45 x 45 cm metal wire racks and dried in an incubator (Gallenkamp/Sanyo) at 35 °C for 12 h. A sample of  $20 \pm 0.1$  g was retrieved from S1 and the  $A_w$  was measured. In S1, inoculation was simulated by placing 400 g of almonds inside a 40 x 36 cm polyethylene bag to which 25 mL of distilled water was added. Almonds and water were mixed by hand-shaking the bag for 2 min. A sample was immediately recovered for  $A_w$  measurement. Wet almonds were placed onto 4 sheets of absorbent paper extended inside the metal rack and allowed to dry for 12 h at 35 °C. In S2, inoculation was performed by arranging 400 g of almonds in a 40 x 36 cm polyethylene bag to which 25 mL of the *Salmonella* cocktail were added. Almonds and inoculum were mixed by hand-shaking the bag for 2 min. Inoculated almonds were placed onto 4 sheets of absorbent paper extended inside a metal rack and allowed to dry for 12 h at 35 °C in the same incubator as S1. After the drying period, S1 and S2 were transferred to separate glass desiccators, which were maintained inside an incubator (Gallenkamp/Sanyo) at 25 °C. To ensure that the  $A_w$  of the almonds was maintained at 0.60, a glass bowl containing a saturated solution of NaBr (Mallinckrodt Baker, Inc., Phillipsburg, N.J.) was placed inside each desiccator. Inoculated almonds were maintained inside the desiccator, before they were removed to be weighted out and treated.

### *Electron beam irradiation*

In order to obtain the most precise dose reading, the depth of the sample was minimized by vacuum packaging  $10.0 \pm 0.2$  g of inoculated almonds on a single-layer-configuration. All samples were heat sealed following an established protocol, established by the Agricultural Engineering Laboratory, to prevent pathogen contamination at the irradiation facility. Inoculated samples were randomly assigned to treatments. Target doses were 0.25, 0.50, 0.75, 1.0 and 1.25 kGy. All assays were carried out with 3 repetitions on two different days. Irradiation treatments were applied using a 1.35 MeV Van de Graaff electron accelerator Type AK model S (High Voltage Engineering Corporation, Burlington, Mass.) managed by the Biological and Agricultural Engineering department, and located in the Hobgood Building at TAMU. Almond packages were secured with tape (3M, St. Paul, Minn.) on an acrylic sheet and positioned approx. 10 cm across the electron gun. The equipment was calibrated with radiochromic film dosimeters (GEX Corp., Centennial, Colo.) and the beam calibration number (BCN), or the applied dose per count, was  $2.07 \times 10^{-6}$  kGy/count.

Due to the low penetration capabilities of the beam, the irradiation treatment of the inoculated almonds was performed on a dual beam configuration manner. Irradiation was first applied on the top surface of the sample, then the equipment was stopped, the sample was inverted, and the bottom surface was treated. This type of configuration was selected based on the MCS subsequently described. Following irradiation, samples were transported in an insulated cooler with refrigerant packs to the Food Microbiology Laboratory for microbiological analysis.

To verify the penetration profile of a single-layer of almonds, MCS was carried out for this configuration using the MCNP ver. 5 software, (General Monte Carlo N-Particle Transport Code) developed by the X-5 Monte Carlo Team from the Diagnostics Applications Group at Los Alamos National Laboratory. The simulator was run in a 4 parallel computer platform at the Department of Biological and Agricultural Engineering at TAMU. The length, width and height dimensions of 40 Nonpareil almonds were measured with a caliper (Scienceware®, Pequannock, N.J.). The almond density was determined using a HD-350E x-ray computed tomography (CT) scanner (Universal Systems Inc.; Solon, Ohio). The geometry of an almond kernel was assumed to be an ellipse and it was built in the MCNP code with the previously mentioned dimensions and the SQ (ellipsoid surface card of MCNP) card function. The ellipsoid was divided into 18 vertical slices and 8 horizontal slices, to obtain 144 tallies. The source card was set to deliver 1.35 MeV. The material composition card was defined with mass fractions of carbon, hydrogen, oxygen, nitrogen and calcium calculated from water (4.7 %), protein (21.22%), total lipid (49.42%), carbohydrate, by difference (21.67%) and ash (2.99%). The proximal composition of almonds, (*Prunus dulcis*), is reported in the USDA National Nutrient Database for Standard Reference (USDA 2009). Finally, the number of source particles was set to 1 million particles.

The MCNP simulation output provided energy data, which was transformed to dose (kGy) by the following method:

$$\text{Dose} \left[ \frac{\text{MeV}}{\text{g}} \right] = \frac{\text{Energy} [\text{MeV}]}{\text{mass} [\text{g}]} \quad \text{Equation 1}$$

$$\text{Mass} [\text{g}] = \rho \left[ \frac{\text{g}}{\text{cm}^3} \right] \times \text{Volume} [\text{cm}^3] \quad \text{Equation 2}$$

$$\text{Dose} [\text{kGy}] = \text{Dose} \left[ \frac{\text{MeV}}{\text{g}} \right] \times \frac{1000\text{g}}{\text{Kg}} \times \frac{1.6022 \times 10^{-13} \text{J}}{1\text{MeV}} \times \frac{1\text{kGy}}{1000\text{Gy}}$$

### *Blanching*

A circulating water bath (Polyscience, Niles, IL.) with a 30 x 25 x 15 cm reservoir was filled with distilled water and heated up to  $88 \pm ^\circ\text{C}$ . Temperature was monitored with a calibrated type K thermocouple (Traceable® Calibration Control Company; Friendswood, Tex.). A temperature-control almond was constructed by wrapping a type K thermocouple terminal around the surface of an almond. This device was used to monitor the temperature on the surface of the almonds throughout the heat treatment. By using the control almond, it was assumed that the temperature on the surface of the control almond represented the temperature of all the almonds during treatment. Samples of  $10.0 \pm 0.2$  g of inoculated almonds were placed in a 12 x 9.5 x 9.5 cm basket along with the temperature-control almond and were submerged into the water bath, one sample at a time. A 4-channel alarm timer (Traceable®) was started when the temperature reached  $88 \pm 2 ^\circ\text{C}$  on the temperature-control almond. After 10, 20, 30, 40 and 50 s, the basket with the almonds was shaken for 3 s, to remove the excess water, and the almonds were immediately transferred to a sterile stomacher bag containing ice-chilled PW. The bag then was submerged into an ice-slurry for at least 5

min. Alongside, positive and negative control samples were carried out. Samples then were collected, and subjected to microbiological analysis.

#### *Oil roasting*

A circulating oil bath (Polyscience) with a reservoir of 30 x 25 x 15 cm was filled with vegetable oil (Great Value, Walmart; Bentonville, Ark.), and heated to  $127 \pm 2$  °C. Temperature was monitored with thermocouples.

Samples of  $10.0 \pm 0.2$  g of inoculated almonds and a temperature-control almond were placed in a 12 x 9.5 x 9.5 cm basket along with a type K thermocouple and submerged into the oil. A timer was started when the temperature-control almond reached  $127 \pm 2$  °C. After 10, 20, 30, 40 and 50 s, the basket was shaken for 3 s, to remove excess oil, and the almonds were immediately transferred to a stomacher bag containing ice-chilled PW and immersed into an ice-slurry for at least 5 min. Alongside, positive and negative control samples were carried out. Samples were then collected for microbiological analysis.

#### *Microbiological analysis*

All samples collected after irradiation, blanching, and oil roasting treatments were pummeled in a Stomacher Lab Blender 400 (Model BA6021, A. J. Seward, London, UK) for 1 min, and appropriate 10-fold serial dilutions plated onto MTSAR. Plates were incubated at 35 °C for 24 h and the colonies were enumerated using a Quebec colony counter. Colonies with a black precipitate on MSTAR were counted as *S.*



Enteritidis rif<sup>+</sup>, and colonies without a black precipitate were recorded as *S. Senftenberg* rif<sup>+</sup>. Colony counts were reported as log CFU/g. All assays were carried out with 3 repetitions on two different days.

**Establishment of the parameters required to achieve a 4-log reduction of a bacterial cocktail containing *S. Enteritidis* rif<sup>+</sup>, and *S. Senftenberg* rif<sup>+</sup> in a commercial electron beam irradiation facility**

Commercial runs require packing almonds by bulk. However, the penetration of an irradiation treatment is limited by the density and the thickness of the product (Hayes and others 1995). Therefore, the penetration profile of the actual commercial packing box was assessed by two methods, Monte Carlo simulation and alanine-dosimeters-dose-mapping.

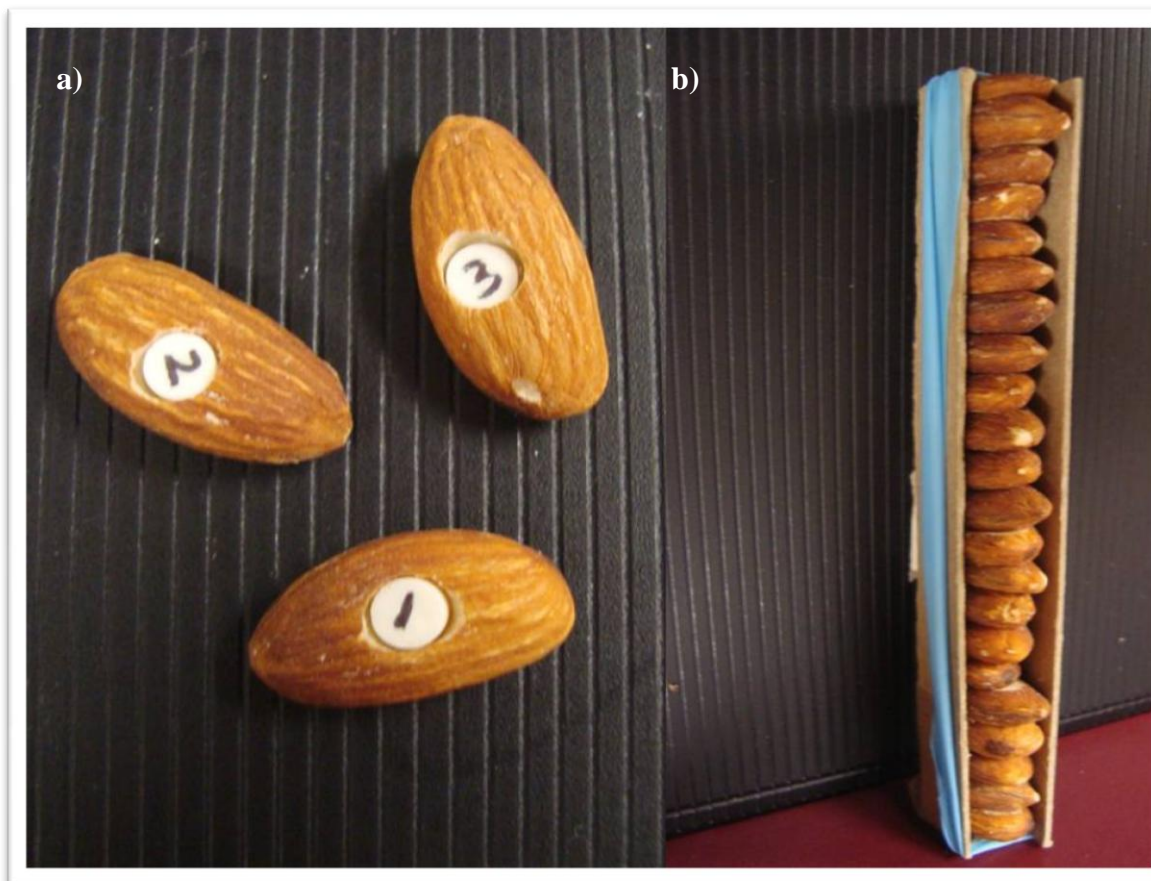
*Monte Carlo simulation*

According to Micke (1996), almonds are carried in wooden bins that hold approx. 900 Kg and repackaged in 22.7 Kg (57 x 40 x 12.7 cm) cardboard boxes for further distribution. A cardboard box of 12.7 x 12.7 x 12.7 cm was filled with Nonpareil almonds and a CT scan was performed with a HD-350E x-ray CT scanner, from where six slices of 0.5 cm each were digitally extracted. Geometry and density data obtained from the CT scan was entered into the MCNP ver.5 software and run in a 4 parallel computer platform. The source card was set to deliver 10 MeV. Input for the material composition card was the same as described in previous section. The geometry was

made up of voxels (three dimensional elements), with a volume of  $3.85 \times 10^{-3} \text{cm}^3$ . As a result of this experiment, a detailed dose profile throughout the entire contents of the box was obtained. This experiment and the data analysis were achieved by collaboration with personnel of the Department of Biological and Agricultural Engineering at Texas A&M University.

#### *Dose-mapping using alanine dosimeters*

To identify the absorbed doses all across the interior space of the box, a dose map of a cardboard box (12.7 x 12.7 x 12.7 cm) filled with Nonpareil almonds was created. Irradiation treatments were carried out at the National Center for Electron Beam Food Research (NCEBFR) at TAMU. To obtain a dose scheme, 3 sets of alanine pellets (GammaService, Radeberg, Germany), were numbered and distributed along the box. Almond dose tracers were assembled by drilling a hole of the size of an alanine dosimeter (diameter = 4.8 mm, radius = 2.9 mm) into the flesh of the almonds to fit a dosimeter in the middle of the kernel. Then, a stack of 23 dose tracers was placed in the center of the box, another set with 6 dose tracers was tightened horizontally, and a third set of 12 dose tracers was tightened diagonally (Figures 1-3). The box was filled with almonds and closed tightly to ensure that almonds did not move when flipping the box. The box was placed onto the conveyor belt, and passed through the electron beam at 5.2 m/min. The effect of dual beam was simulated by inverting the box once. Alanine pellets were read using an electron paramagnetic resonance (EPR) spectroscope (Bruker EMS 104 EPR Analyzer, Bruker Instruments, Germany).



**Figure 1-a) Alanine dosimeters inserted into almond kernel's flesh. b) Vertical stack of almond kernels with alanine dosimeters inserted into the flesh.**



**Figure 2-Alanine dosimeters fixed in a horizontal, vertical and diagonal configuration inside a 12.7 x 12.7 x 12.7 cm cardboard box.**



**Figure 3-Alanine dosimeters fixed in a horizontal, vertical and diagonal configuration inside a 12.7 x 12.7 x 12.7 cm cardboard box filled with almonds.**

### **Evaluation of the chemical and sensory quality of almonds commercially processed with electron beam irradiation, blanching, and oil roasting**

For practical reasons the food industry frequently depends on accelerated shelf-life test (ASLT) techniques to reduce the process of assessing the shelf life of a product, especially when the shelf-life of the product is lengthy. ASLT can be applied to any food deterioration system that aligns with a kinetic model. Most food deterioration systems align with zero and first order kinetic models (Lee and others 2003). According to Labuza (1985) a linear model can be applied to describe the rate of a reaction at different temperatures. The use of high temperatures is the most convenient way of accelerating the rate of a deterioration reaction (Mizrahi 2000). In order to assess the quality decay of blanched, roasted and irradiated almonds an ASLT was carried out in the present study.

To observe deterioration of quality attributes for at least one half of the industry's shelf-life, the following estimations were prepared. According to industry standards, the estimated shelf-life of raw almonds is about 24 mo when stored at 25°C (Blue Diamond 2010). Tan and others (2001) calculated the activation energy ( $E_a$ ) of 10 different oxidized vegetable oils, finding them to range between 79-104 kJ/mol. Based on this numbers, the  $E_a$  for the lipid oxidation of almonds was assumed as the mean of the oils studied by Tan and others (2001), at 90 kJ/mol. This estimated almond  $E_a$  (calories/mol) and the temperature (T) in °K was substituted into equation 3 (Ragnarsson and Labuza 1977) to obtain the degradation rate in 10 °C intervals, also called  $Q_{10}$ . The unit conversion used was 238 calories/kJ.

$$\log Q_{10} = \frac{2.189E_a}{(T + 10)T} \quad \text{Equation 3}$$

The  $Q_{10}$  value was determined to be 2.7 at 50 °C, concurring with similar values reported by Taokis and Labuza (2000). The Arrhenius model, or  $Q_{10}$  is commonly used to estimate the reaction rate if the food product is held at a different temperature (Labuza and Schmidl 1985). In the present study, we wanted to simulate the conditions that would be equivalent to one-half of the standard shelf life of almonds at 25 °C ( $\theta_{25}$ ) (12 mo) if the almonds were stored at 50°C. Therefore, equation 4 (Labuza and Schmidl 1985) was solved for the shelf life at 50 °C ( $\theta_{50}$ ), with a temperature difference ( $\Delta T$ ) of 25 °C.

$$\Theta_{25} = \Theta_{50} Q_{10}^{\frac{\Delta T}{10}} \quad \text{Equation 4}$$

$$\Theta_{50} = \frac{12 \text{ mo}}{2.7^{2.5}} = 1 \text{ mo}$$

Therefore, the study was set to last for 1 mo at a storage temperature of 50 °C. Almonds were stored in a temperature controlled chamber (Model: 1350 GM, Sheldon Manufacturing, Inc., Cornelius, Ore.) at 50 °C where temperature and relative humidity (RH) were monitored with a wireless digital thermometer and hygrometer device (Springfield ® Precise Temp™, Columbus, Nebr.). Samples were retrieved from the chamber every wk for to be tested for free fatty acids (FFA), peroxide value (PV), and 2-thiobarbituric acid reactive substances (TBARs).

#### *Irradiation of raw almonds*

Based on the irradiation challenge studies results, the D-value of *S. Enteritidis* PT 30 was set at 0.9 kGy. Therefore, to achieve a 4 log reduction of *S. Enteritidis* PT 30

from almonds, and comply with the Final Rule for the Mandatory Pasteurization of California Almonds the minimum target dose was 3.6 kGy. On two separate days, three cardboard boxes (12.7 x 12.7 x 12.7 cm) were filled with raw Nonpareil almonds in the Food Sensory Laboratory. The boxes were tightly closed and transported to the National Center for Electron Beam Food Research. Calibration of the conveyor belt was performed by using a speed-check box, a dummy box filled and packed the same way as the sample boxes. To ensure that the dose read on these dosimeters represented the dose absorbed by the top and bottom layer of almonds, a dent of 0.5 x 0.5 cm was carved into each of the irradiated boxes. An alanine dosimeter protected by a polyethylene bag was placed inside each dent and secured with tape. Using the speed-check box, speed adjustments to the conveyor belt were made until the target dose of 3.6 kGy was achieved at the top and bottom of the speed-check box.

Aiming to a more uniform dose distribution throughout the box contents, the treatment was applied on a simulated dual beam configuration. Each box was passed through the e-beam source twice, with one inversion between each pass. To monitor the absorbed dose, an alanine pellet was placed at the top and at the bottom of each of the sample boxes. Each dosimeter measured the entrance dose (from pass 1 and 2) plus the exit dose, if any. After the irradiation treatment was applied, samples were brought back to the TAMU Sensory facility for quality assessment.



*Sensory analysis*

In this study there were 3 types of commercially treated almonds, irradiated, blanched and roasted. A consumer sensory panel was carried out at the sensory booths of the TAMU Sensory facility on the same day that almonds were irradiated. Blanched almonds were skinless, which made the differences in color evident; therefore, white fluorescent overhead lights were used. Each of the three treatments was assigned a random three digit code, and sample order was randomly assigned for each consumer. Every consumer received a labeled plastic weigh boat, with two almond kernels, a cup of double distilled, deionized water, and two unsalted crackers as palette cleansers. Samples were distributed one at a time, and consumers were instructed to use crackers as palette cleansers between samples. Each consumer received a package that included one ballot per sample, a consumer preference form and a demographic questionnaire. On the sample ballot, consumers were asked to rank in a 9-point hedonic and intensity scales the following attributes, overall like/dislike, like/dislike of the exterior color, like/dislike of the interior color, level of texture, like/dislike of the almond flavor, level of bitterness, level of rancidity, and likeliness of purchase. In the preference test form, consumers were asked to select the sample that they preferred overall, for interior color, for exterior color, for texture, for almond flavor, considered less bitter and considered less rancid. The last page of the package asked demographic information, such as age and gender. Additionally, four questions to assess frequency of consumption, lifestyle, and consumer perception towards the treatment of nuts, were included.

*Gas chromatography and spectroscopy profile of raw and irradiated almonds*

To further investigate the impact of e-beam irradiation on the quality of raw almonds, an aroma flavor profile of raw and irradiated almonds was performed. These tests, which included full aroma/flavor profiles by gas chromatography/mass spectrometry/olfactometry (GCMS-O) analysis, were outsourced to Microanalytics™ (Round Rock, TX.). Immediately after samples were received at the third-party laboratory facilities, an overall odor evaluation of the raw and irradiated almonds was performed by trained analysts.

To determine the nature of the compounds responsible for the aroma/flavor profiles of raw and irradiated almonds, volatiles expelled from each sample were captured in the following manner. One hundred and twenty-five g of raw and irradiated almonds were individually placed in clear glass quart jars fitted with Teflon lined lids designed for solid phase microextraction (SPME) sample collection. Jars headspace was equilibrated at 25 °C for approximately 24 h. After equilibration, a SPME fiber was inserted through a pinhole in the lid and exposed to the headspace volatiles. The headspace collection was carried out at 25 °C. The type of SPME fibers used for collection was Carboxen/PDMS SPME fiber (StableFlex™ 85µm, Supelco PN 57334-U).

The SPME collected volatiles were thermally desorbed in the gas chromatograph (GC) inlet at 250 °C and analyzed using the Microanalytics™ Aromatrx™ GC/MS/Olfactometry integrated system operated under the following instrument parameters: The Aromatrx™ multidimensional gas chromatography system consists of

an high performance (HP) 6890 GC coupled to an HP mass spectrometry detector (MSD). The injection mode was splitless with an inlet fitted with a Merlin MicroSeal septum. The inlet temperature was 250 °C. There were three detectors; the first one used flame ionization with a temperature of 300 °C, the second detection was obtained by an electron impact mass spectrometer, and the third detection point was set with a sniff port, at a temperature of 220 °C. The GC system was set up with two columns, a 30 m by 0.53 mm internal diameter (ID), DB 5MS – 0.25µm film column was used as the pre-column, and a 30 m by 0.53mm ID, Solgel-Wax – 0.1µm film as the analytical column. The oven was programmed to initiate at 40 °C, with a 3 min hold, followed by a 7 °C /min increase, until it reached the end temperature of 240 °C, where a 8.4 min hold was arranged.

From detector 1 and 2, GC/MS chromatograms of the headspace of each sample were obtained. Simultaneous to the construction of the corresponding GC/MS chromatograms, aromagrams were generated. From detector 3, the sniff port, aroma characters were detected, classified and ranked by a trained analyst. Descriptors and relative intensities of these aroma characters were recorded to construct aromagrams. Aromagrams of raw and irradiated samples were recorded using the AromaTrax™GC/MS/Olfactometry integrated system. Aromagrams are the graphical representation of a sample's odor intensity versus retention time in the GC/MS chromatogram format. The aroma characters were matched with the corresponding GC/MS chromatographic responses and the tentative mass spectral identification was

obtained from matching the measured spectrum with the general mass spectral data base (Wiley Mass Spectral Library, Hoboken, N.J.).

#### *Quality of fatty acids*

The experimental design consisted of three treatments where each treatment contained 3 repetitions. The entire experiment was performed twice (two trials). Almonds from each treatment, repetition and trial were retrieved from the storage incubator every week for 4 wk. As soon as the samples were obtained, they were frozen at -20 °C. Once all the samples were collected, 8 random samples were chosen every testing d.

#### *Oil extraction*

A 60-g sample of frozen almonds was ground in a commercial blender (Model 51BL32 Waring; Torrington, Conn.) for 1 min. Then, the almond powder was transferred to a 250 mL Erlenmeyer flask, to which a 100 mL of 95% hexane (Mallinckrodt Baker) were added. The Erlenmeyer flask was placed into a water bath shaker (Model G-76, Brunswick Scientific, Edison, N.J.) at 40 °C for 3 h.

Semi-defatted samples, oil and solvent were filtered in a Buchner funnel using grade No. 1 Whatman filter paper (GE, Fairfield, CT) of 12.5 cm in diameter. The oil and solvent mixture was transferred to a round-bottom-flask and placed in a rotavapor (Mod 315686, Brinkmann Instruments, Riverview, Fla.) at 50 °C for 5 min, until all the

hexane was evaporated. Finally, nitrogen (Brazos Valley Welding Supply, Bryan, Tex.) was flushed into the flask to remove any remaining solvent.

#### *Percentage of free fatty acids*

This procedure follows the American Oils Chemists' Society Method (AOCS 1973) Ca 5a-40. Immediately after the oil extraction was finished, a subsample of  $5 \pm 0.1$  g of oil was weighted in a 250 ml Erlenmeyer flask, and 50 ml of 95% neutralized ethanol was added. One ml of phenolphthalein 1% indicator solution (BDH Merck Ltd.) was added to the mixture and stirred with a magnet over a magnetic stirrer platform. Then, titration was performed with 0.1 N sodium hydroxide solution (Fisher, Pittsburgh, Pa.) until a permanent pink color was achieved.

Calculations of % of free fatty acids (%FFA) were obtained as follows:

$$\% \text{FFA of oleic acid} = \frac{\text{mL of NaOH} \times 0.1 \times 28.2}{\text{weight of the sample}} \quad \text{Equation 5}$$

#### *Peroxide value*

This procedure follows the AOCS (1973) Cd 8-53 method. A subsample of  $5 \pm 0.1$  g of oil was weighed into a 250 mL Erlenmeyer flask with cap. Fifty mL of a 3:2 mixture of glacial acetic acid (Mallinckrodt Chemicals) and iso-octane (Mallinckrodt Chemicals) and 0.5 ml of a saturated potassium iodide (Sigma-Aldrich®) solution were added to the oil and vigorously shaken for 1 min. Then, 30 mL of deionized water and 0.5 mL of 1% (w/v) starch indicator (Ricca Chemical Company, Arlington, Tex.) were

mixed with the sample. Titration with 0.01 sodium thiosulfate ( $\text{Na}_2\text{S}_2\text{O}_3$ ) (BDH Merck Ltd.) was performed until the gray-brown color of the mixture turned milk-like white.

Calculations of PV were obtained as follow:

$$\text{PV} = \frac{\text{mL of Na}_2\text{S}_2\text{O}_3 \times 0.01 \times 1000}{\text{weight of the sample}} \quad \text{Equation 6}$$

#### *2-thiobarbituric acid reactive substances*

This procedure follows the AOCS (1973) Cd 19-90 method. A subsample of 200  $\pm$  0.5 mg of oil was weighted into a 25 mL volumetric flask. Then, 1-butanol (Mallinckrodt Chemicals) was added up to the 25 mL mark. The mixture was shaken and 5 mL were transferred to a screw cap tube. A 2-thiobarbituric acid mixture was prepared by weighing out 200 mg of 2-thiobarbituric acid (Sigma-Aldrich<sup>®</sup>) in a 100-mL-volumetric flask and making up the volume with 1-butanol. Five ml of the 2-thiobarbituric acid mixture were added to the screw cap tube with the sample. A blank was made by adding 5 mL of 1-butanol plus 5 mL of the 2-thiobarbituric acid mixture. Sample tubes and blank were shaken and placed in a water bath (Model: WB111OH-1, Lindberg/Blue M; Asheville, N.C.) at 90 °C for 2 h. Tubes were removed from the water bath and let to temper for 10 min in a plastic tub with cool tap water. Finally, tube contents were transferred to disposable cuvettes and read at 530 nm in a General Purpose UV/Vis spectrophotometer (DU<sup>®</sup> 520, Beckman Coulter Inc., Brea, Calif.). Calculations of TBARs were obtained as follow:

$$\text{TBAR} = \frac{50 \times (\text{Absorbance of test} - \text{Absorbance of blank})}{\text{weight of the sample (mg)}} \quad \text{Equation 7}$$

*Statistical analysis*

Growth characteristics of ATCC strains and rif<sup>+</sup> strains were compared by plotting O.D. values as a function of time. Growth data were fitted to a Baranyi model equation, using the MicroFit v1.0 software. For individual growth curves, the following growth parameters were estimated: initial population ( $N_0$ ), maximum population density ( $N_{max}$ ), maximum specific growth rate ( $\mu_{max}$ ), lag phase time (t-lag), and doubling time (t-d). Growth parameters of each strain were compared using the ANOVA procedure of SPSS (Statistical Package for the Social Sciences, IBM, Somers, NY). An alpha of 0.05 was used to determine significance.

In the thermal and irradiation resistance experiments, the bacterial survival data were transformed to log CFU/g. Survivors data were plotted as a function of time or dose, respectively, and fitted to a linear model equation using SPSS. Individual D-values were calculated as the reciprocal of the slope of the linear model. Comparisons of thermal and irradiation resistance between strains were conducted by applying the slope comparison procedure on the regression lines, using the general linear model of SPSS. An alpha of 0.05 was used to determine significance.

Multiple comparison procedures were conducted to determine differences among means when comparing consumer sensory data. Data were tested for normality using the box-cox test of SAS (Statistical Analysis Systems Institute, Cary, N.C.), and transformed when the  $\lambda$  value was different from 1.0. Sensory attributes were analyzed with the general linear model (PROC GLM) procedure of SAS to determine significance between irradiation, blanching and oil roasting treatments. When means were different, they were

separated with least squared means (LSM) procedure. An alpha of 0.05 was used to determine significance.

To determine differences among means when comparing chemical tests, multiple comparison procedures were conducted. To determine significance between irradiation, blanching and oil roasting treatments, across storage time, data were analyzed with the general linear model (PROC GLM) procedure of SAS. Irradiated, blanched and oil roasted almonds, from the initial batches, were subsampled to perform chemical analysis through time. Thus, a PROC MIXED repeated measures model from SAS was recommended to analyze these data. The proc mixed repeated measures model had almost the same sensitivity as the PROC GLM test and the inferences were no different. Therefore, PROC GML was selected to analyze these data. When means were different, they were separated by the least squared means (LSM) procedure. An alpha of 0.05 was used to determine significance.



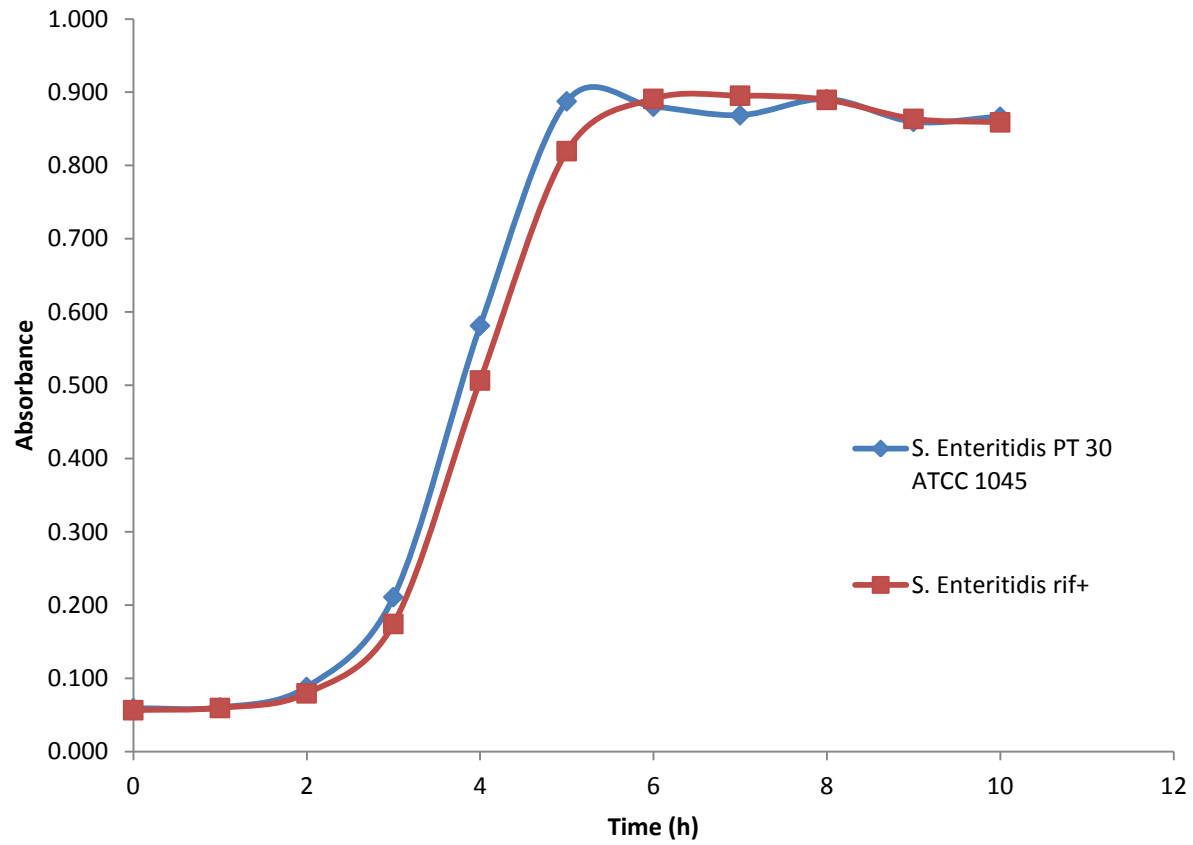
## RESULTS AND DISCUSSION

### Preliminary studies

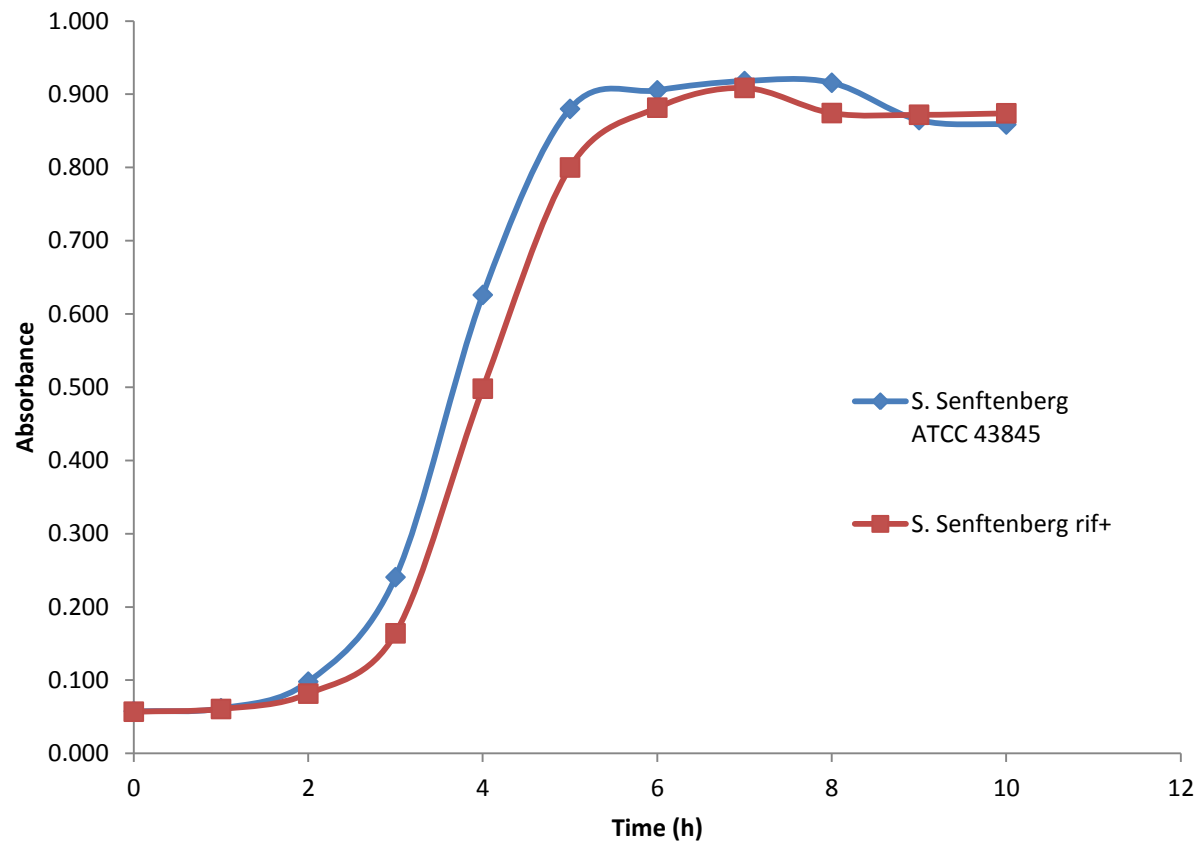
#### *Growth characteristics*

The growth curves of *S. Enteritidis* PT 30 ATCC 1045 and its rif<sup>+</sup> derivative are presented in Fig. 4. Figure 5 presents the growth curves of *S. Senftenberg* ATCC 43845 and its rif<sup>+</sup> derivative. These curves indicate that the growth characteristics of these organisms and their rif<sup>+</sup> derivatives are very similar. Nonetheless, growth curves were fitted to a Baranyi regression equation, to quantitatively compare growth parameters between strains.

The growth parameters extracted from the Baranyi model were: the initial ( $N_0$ ), and final ( $N_{\max}$ ) bacterial cell density expressed as optic density, maximum specific growth rate ( $\mu_{\max}$ ), lag time (t-lag), and doubling time (t-d) for *S. Enteritidis* PT 30 ATCC 1045, *S. Senftenberg* ATCC 43845, and their rif<sup>+</sup> derivatives are presented in Tables 1 and 2. There were no differences ( $P > 0.05$ ) for any of these growth parameters between *S. Enteritidis* PT 30 ATCC 1045 and its rif<sup>+</sup> derivative. Comparisons between *S. Senftenberg* ATCC 43845 and its rifampicin derivative showed no differences between the strains for  $N_0$ ,  $N_{\max}$ , and, t-lag, while the  $\mu_{\max}$ , and t-d were significantly different ( $P < 0.05$ ). The mean and SD for the  $\mu_{\max}$  of *S. Senftenberg* ATCC 43845 and its rif<sup>+</sup> variant were  $2.2 \pm 0.08 \text{ h}^{-1}$  and  $1.8 \pm 0.12 \text{ h}^{-1}$ , respectively. The mean and SD of the t-d for this organism and its rif<sup>+</sup> variant were  $0.31 \pm 0.01 \text{ h}^{-1}$ ,  $0.38 \pm 0.03 \text{ h}^{-1}$ . The



**Figure 4-Growth curves of *S. Enteritidis* PT 30 ATCC 1045 and *S. Enteritidis* rif+ in tryptic soy broth at 37 °C.**



**Figure 5-Growth curves of *S. Senftenberg* ATCC 43845 and *S. Senftenberg* rif+ in tryptic soy broth at 37 °C.**

**Table 1-Growth parameters for ATCC and rif+ *Salmonella* Enteritidis strains in tryptic soy broth at 37 °C.**

Microorganism	Mean <sup>a</sup> ± SD <sup>b</sup>				
	N <sub>0</sub> <sup>c</sup> (O.D.)	N <sub>max</sub> (O.D.)	μ <sub>max</sub> (h <sup>-1</sup> )	t-lag (h)	t-d (h)
<i>S. Enteritidis</i> PT30 ATCC 1045	0.06 ± 0.01 A <sup>c</sup>	0.90 ± 0.04 A	2.08 ± 0.17 A	3.16 ± 0.22 A	0.33 ± 0.03 A
<i>S. Enteritidis</i> rif+	0.07 ± 0.01 A	0.90 ± 0.04 A	2.03 ± 0.15 A	3.38 ± 0.15 A	0.34 ± 0.03 A

N<sub>0</sub>: initial bacterial cell density; N<sub>max</sub>: final bacterial cell density; μ<sub>max</sub>: maximum specific growth rate; t-lag: lag time; t-d: doubling time; O.D. :optical density

<sup>a</sup> Mean values were obtained from two independent replicates

<sup>b</sup> Standard deviation

<sup>c</sup> Means in the same column with the same letter (ABC) are not significantly different ( $P > 0.05$ )

**Table 2-Growth parameters for ATCC and rif+ *Salmonella* Senftenberg strains in tryptic soy broth at 37 °C.**

Microorganism	Mean <sup>a</sup> ± SD <sup>b</sup>				
	N <sub>0</sub> <sup>c</sup>	N <sub>max</sub>	μ <sub>max</sub>	t-lag	t-d
	(O.D.)	(O.D.)	(h <sup>-1</sup> )	(h)	(h)
<i>S. Senftenberg</i> ATCC 43845	0.07 ± 0.01 A	0.90 ± 0.02 A	2.20 ± 0.08 A	3.20 ± 0.03 A	0.31 ± 0.01 A
<i>S. Senftenberg</i> rif+	0.09 ± 0.02 A	0.93 ± 0.05 A	1.81 ± 0.12 B	3.40 ± 0.19 A	0.38 ± 0.03 B

N<sub>0</sub>: initial bacterial cell density; N<sub>max</sub>: final bacterial cell density; μ<sub>max</sub>: maximum specific growth rate; t-lag: lag time; t-d: doubling time; O.D.: optical density

<sup>a</sup> Mean values were obtained from two independent replicates

<sup>b</sup> Standard deviation

<sup>c</sup> Means in the same column with the same letter (ABC) are not significantly different ( $P > 0.05$ )

differences in these two parameters indicate that the rif<sup>+</sup> strain grows at a slower rate than the non-rif<sup>+</sup> strain. However, both strains reached the same  $N_{\max}$ .

Overall, the similarities in growth characteristics between the rif<sup>+</sup> and the non-rif<sup>+</sup> strains suggest that the mutation for rifampicin resistance resulted in minimal, if any, compromise to the reproductive capabilities of these strains. Therefore the rif<sup>+</sup> strains selected for this study are suitable for use in the challenge studies presented in the present work.

#### *Thermal resistance of rifampicin resistant Salmonella strains*

Thermal resistance experiments were conducted to determine if *S. Enteritidis* PT 30 ATCC 1045, and *S. Senftenberg* ATCC 43845 showed similar behavior when exposed to heat, than their rif<sup>+</sup> derivatives. Almonds were inoculated with each microorganism, and subjected to blanching at 88 °C for 60 s. The mean and SD log reduction of *S. Enteritidis* PT 30 ATCC 1045 inoculated onto almond surfaces was  $4.2 \pm 0.7$  CFU/g, and the mean and SD log reduction of the rif<sup>+</sup> *S. Enteritidis* was  $4.2 \pm 0.4$  CFU/g. There were no significant differences ( $P > 0.05$ ) between the log reductions of these two strains. The mean and SD log reduction of *S. Senftenberg* ATCC 43845 inoculated onto almond surfaces was  $4.3 \pm 0.4$ , and  $4.8 \pm 0.5$  CFU/g for the rif<sup>+</sup> *S. Senftenberg*, with no significant differences ( $P > 0.05$ ) between these two strains (data not presented in tabular form).

*Radiation resistance of rifampicin resistant Salmonella strains*

Radiation resistance experiments were conducted to ascertain if the rif<sup>+</sup> derivatives of *S. Enteritidis* PT 30 ATCC 1045 and *S. Senftenberg* ATCC 43845 showed similar resistance to radiation as their parent strains. Agar plugs were inoculated with each type of strain and exposed to e-beam irradiation at a dose of 1.4 kGy. The mean and SD log reductions of *S. Enteritidis* PT 30 ATCC 1045 and its rif<sup>+</sup> derivative were  $3.0 \pm 0.1$  and  $2.9 \pm 0.4$  CFU/g, correspondingly. These values were not significantly different ( $P > 0.05$ ). Similarly, no differences ( $P > 0.05$ ) were observed between the mean and SD log reduction of *S. Senftenberg* ATCC 43845 ( $2.8 \pm 0.1$  CFU/g), and its rif<sup>+</sup> derivative ( $3.0 \pm 0.7$  CFU/g) (data not presented in tabular form).

The similarities observed in growth patterns, thermal, and irradiation resistance of the rif<sup>+</sup> resistant strains and the ATCC strains ensure that the use of rif<sup>+</sup> resistant derivatives will robustly mimic the response of the ATCC strains with the advantages that markers pose in the execution of challenge studies.

*Restoration of almond Aw after storage and inoculation*

In the present study, almonds were removed from the refrigerated storage and the mean and SD of their Aw was  $0.849 \pm 0.010$ . Then, a batch of 420 g of almonds was dried at 35 °C for 12 h, and their Aw dropped to  $0.619 \pm 0.018$ . The next step was to simulate the inoculation process, for which the same batch of dried almonds was mixed with 25 ml of distilled water. The mean and SD of the Aw after this step was  $0.903 \pm 0.013$ . These wet almonds were again dried at 35 °C for 12 h, and their Aw was restored

to  $0.562 \pm 0.010$ . This protocol was the premise under which the challenge studies were carried out in the present work. The inoculated almonds used in the challenge studies of the current work ensured that the pathogen of interest was challenged under the worst-case-scenario conditions. The design and establishment of the protocol to restore the  $A_w$  of inoculated almonds is a groundbreaking method that will impact the reliability of results obtained from challenge studies conducted in low moisture foods.

**Decimal reduction dose and time of *S. Enteritidis* rif<sup>+</sup>, and *S. Senftenberg* rif<sup>+</sup> on inoculated almonds using electron beam irradiation, blanching and oil roasting**

The recent mandatory pasteurization of almonds policy has encouraged the scientific community and the industry to investigate the efficacy of cutting-edge technologies, and to establish critical control points in conventional technologies used in the treatment of raw almonds. During the development of new methods, or the evaluation of current ones, it is paramount to consider *Salmonella*'s increased resistant, when osmotically challenged, to ensure the validity of the studies. Additionally, in the evaluation of widely known technologies, recently applied to almonds, it is essential to consider the impact of the treatment in the quality of the final product and the consumer's perception.

*Irradiation*

The application of irradiation treatments to calculate the D-values of *S. Enteritidis* rif<sup>+</sup>, and *S. Senftenberg* rif<sup>+</sup> were obtained under tight conditions using a

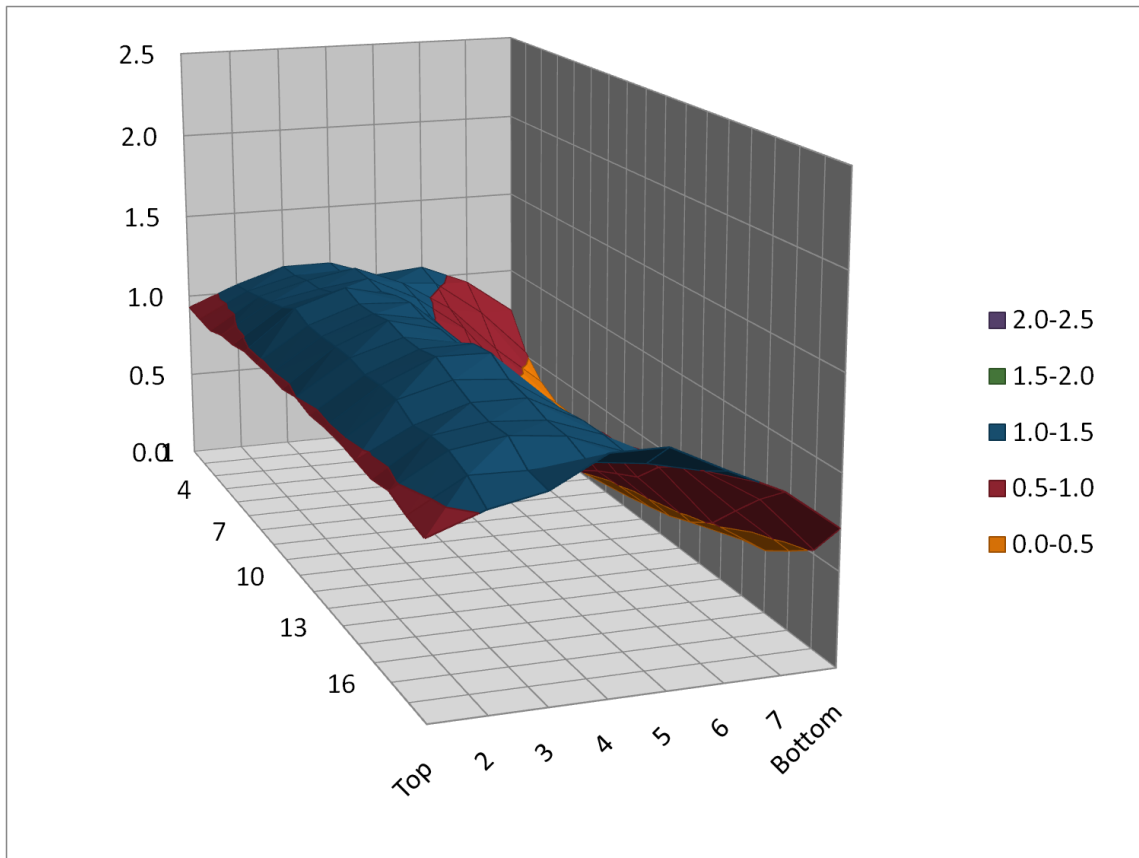


single beam 1.35 MeV Van de Graaf accelerator. Since the estimation of D-values is highly dependent on the correct estimation of the absorbed dose, simulations of single and dual beam configurations were performed in advance.

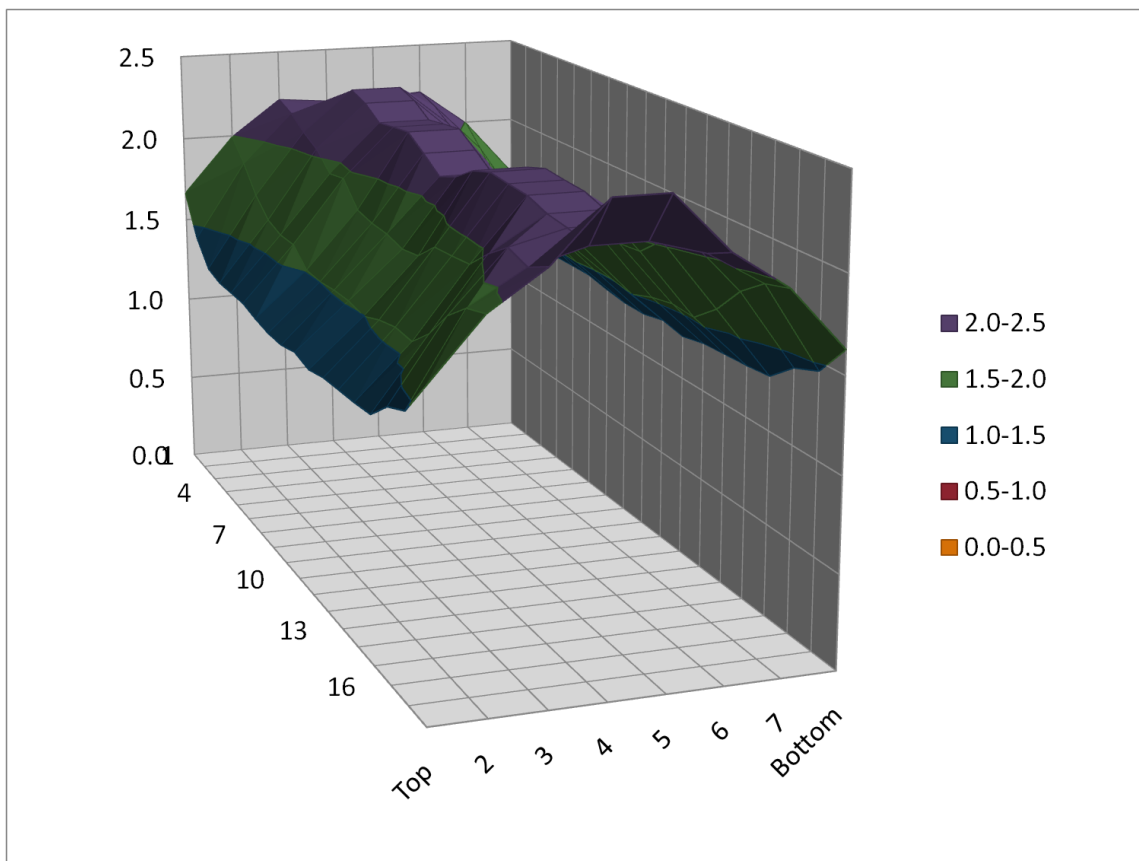
The following analysis was performed under the supervision of Dr. Rosana Moreira and with the assistance of Dr. Jongsoo Kim, from the Department of Agricultural Engineering at TAMU. The absorbed dose distribution throughout the volume of an almond kernel was estimated by Monte Carlo simulation. Input for the surface, cell, energy source, and material composition cards were entered into the Monte Carlo code. For the surface card, an almond kernel was assumed to have a perfectly ellipsoidal geometry, with the mean length ( $2.26 \pm 0.07$  cm), width ( $1.25 \pm 0.04$  cm), and height ( $0.76 \pm 0.05$  cm) of the measurement of 40 Nonpareil almonds, and the almond's density of  $1.037 \text{ g/cm}^2$  obtained in the CT scan procedure of this work.

According to the Monte Carlo simulation applying 1.35 MeV on a single beam configuration produces large dose variations across the volume of an almond's kernel. According to Fig. 6, the bottom section of the kernel only receives 40% of the dose absorbed by the top surface layer, leaving the bottom layer under treated. On the other hand, the core of the kernel receives 120% of the surface dose, meaning that the core is an over treated area. The dose uniformity ratio (DUR) of this configuration was 5.19.

As an alternative to the single beam shortcoming, a dual beam configuration was explored. The Monte Carlo simulation results of the dual beam configuration are shown in Fig. 7. With the use of a dual beam, the DUR was reduced to 1.97. This configuration allowed a inclusive treatment of the top and bottom surfaces of the almond kernel. Dose-



**Figure 6.-Three dimensional dose distribution of a horizontally placed almond kernel irradiated with a Van de Graaff accelerator of 1.35 MeV in a single beam configuration. Doses were estimated by Monte Carlo simulation.**

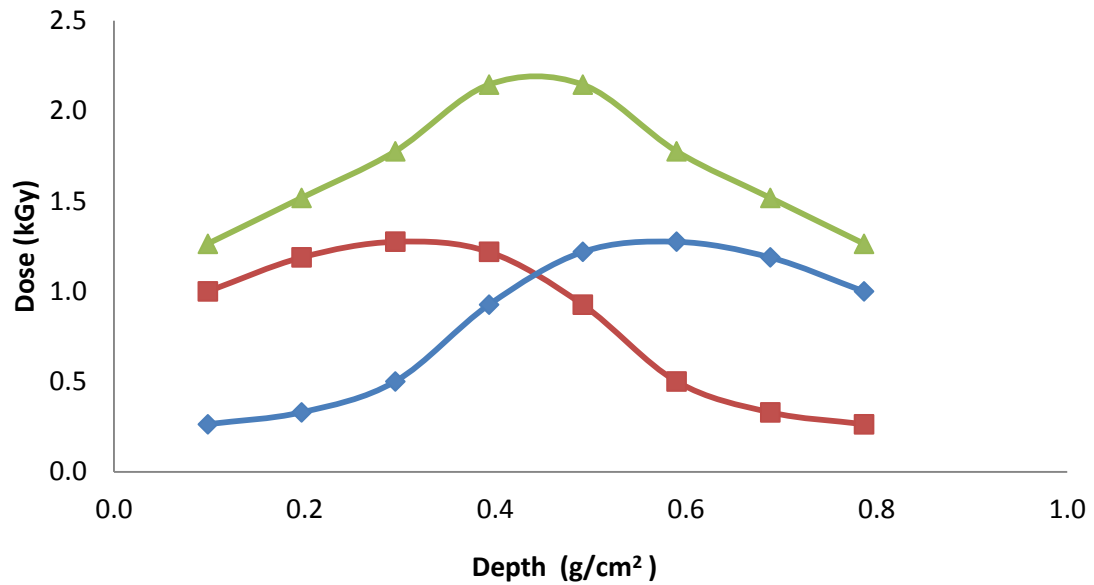


**Figure 7- Three dimensional dose distribution of a horizontally placed almond kernel irradiated with a Van de Graaff accelerator of 1.35 MeV in a dual beam configuration. Doses were estimated by Monte Carlo simulation.**

depth curves of the single and dual beam arrangements are presented in Fig. 8. It is important to make a remark about the higher absorbed doses at the edges, compared to the center of the kernel. Similar findings were reported by Kim and others (2010) when simulating the electron beam irradiation of whole cantaloupes. This phenomenon may be attributed to some electrons scattering at the edges of an irregular product producing surface-edge buildups (Kim 2010).

From these results, dual beam was determined to be the most appropriate configuration for applying an irradiation treatment that would fully cover the top and bottom surfaces of a single layer of almonds. Then, almonds inoculated with *S. Enteritidis* rif<sup>+</sup>, and *S. Senftenberg* rif<sup>+</sup> were irradiated with a 1.35 MeV Van de Graaff accelerator in a dual beam configuration achieved by irradiating one side and then inverting the sample to irradiate the other side. Absorbed doses of the top and bottom surfaces were adjusted based on the results obtained from the Monte Carlo simulation. The mean and SD of the doses applied were  $1.24 \pm 0.001$ ,  $1.00 \pm 0.001$ ,  $0.752 \pm 0.002$ ,  $0.499 \pm 0.001$ ,  $0.250 \pm 0.001$ .

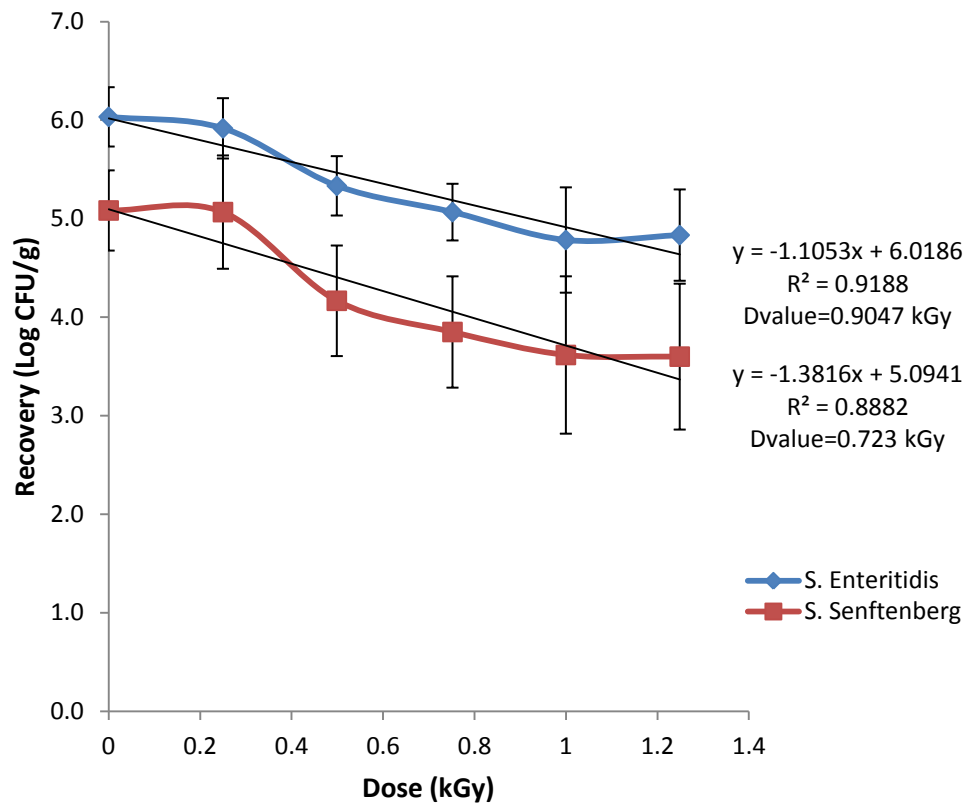
To determine the D-values of the *Salmonella* working strains, irradiation inactivation curves were constructed for each microorganism, and linear regression analysis was applied to the data. From the inactivation curves, D-values were calculated and compared among the two *Salmonella* strains (Fig. 9). The mean and SD D-value for *S. Enteritidis* rif<sup>+</sup> was  $0.90 \pm 0.013$  kGy and for *S. Senftenberg* rif<sup>+</sup> was  $0.72 \pm 0.04$  kGy. Contrasting results were found by Prakash and others (2009), which reported that *S. Enteritidis* PT 30 inoculated onto Nonpareil almonds showed a D-value of 1.25 kGy, a



**Figure 8- Single (red and blue) and double sided (green) dose-depth curves of an almond kernel irradiated with a Van de Graaff accelerator of 1.35 MeV.**

The red line indicates the energy deposition profile of a single beam impacting the top surface of the product, whereas the blue line is the energy trajectory of a beam impacting from the bottom surface.

Doses were estimated by Monte Carlo simulation.



**Figure 9-Mean and standard deviations of the recovery of *S. Enteritidis* rif+ and *S. Senftenberg* rif+ from Nonpareil almonds irradiated at doses delivered with a Van de Graaff accelerator of 1.35 MeV.**

For each strain, recovery means were fitted to a linear equation from where D-values were estimated. *S. Enteritidis* rif+.

value 40% higher than that obtained in the present study. Such large difference in the estimation of *S. Enteritidis* D-value may be attributed to the large variations in energy delivered at commercial irradiation facilities, as performed by Prakash and others (2009).

According to the findings in the present work, to use irradiation as the only intervention to achieve the mandatory 4-log reduction of *Salmonella*, a minimum dose of 3.6 kGy should be applied. Statistical comparison of the slopes of the linear regression curves of each serotype determined that there were no statistical differences ( $P = 0.3360$ ) between *S. Enteritidis* rif<sup>+</sup> and *S. Senftenberg* rif<sup>+</sup>. Therefore, the resistance to irradiation was not significantly different between strains. Even though *S. Senftenberg* was regarded as a heat resistant strain, it does not pose a higher resistance to irradiation than *S. Enteritidis* when inoculated onto almond surfaces.

Sherry and others (2004) compared the thermal, irradiation and high pressure resistance of 40 *Salmonella* serovars in liquid media. The authors concluded that the irradiation resistance of *S. Senftenberg* and *S. Enteritidis* PT 4 was very similar, concurring with this work findings.

Palekar (2004) reported the D-value of *S. Poona* inoculated on the surface of cantaloupe slices to be 0.211 kGy. Prakash and others (2007) documented that the D-value of several serotypes of *Salmonella* inoculated onto diced tomatoes was between 0.25-0.35 kGy. Niemira and others (2001) investigated the irradiation resistance of four *Salmonella* serovars inoculated into orange juice and treated with gamma irradiation, finding that the D-values varied greatly between serovars. *S. Anatum* had the highest D-

value (0.71 kGy), whereas *S. Infantis* had the lowest (0.35 kGy). The large differences in D-values between commodities may be explained by the fact that when *Salmonella* is exposed to high stress environments, such high acidity or low  $A_w$  foods, some proteins are exerted as an osmotic or acid stress response (Cabiscol and others 2000). These same proteins may also contribute to the repair mechanism of *Salmonella* against irradiation, similar to the cross-protection observed by heat shock proteins. This hypothesis is supported by the findings of Mattimore and Battista (1996), and Battista (1997), who suggested that the radiation resistance of *Deinococcus radiodurans* may be an evolutionary adaptation trait that this organism developed to counteract the DNA damage produced by harsh desiccation environments. Cross-protection between stresses may be the reason why osmotically challenged *Salmonella*, inoculated onto almonds, has a greater resistance to irradiation.

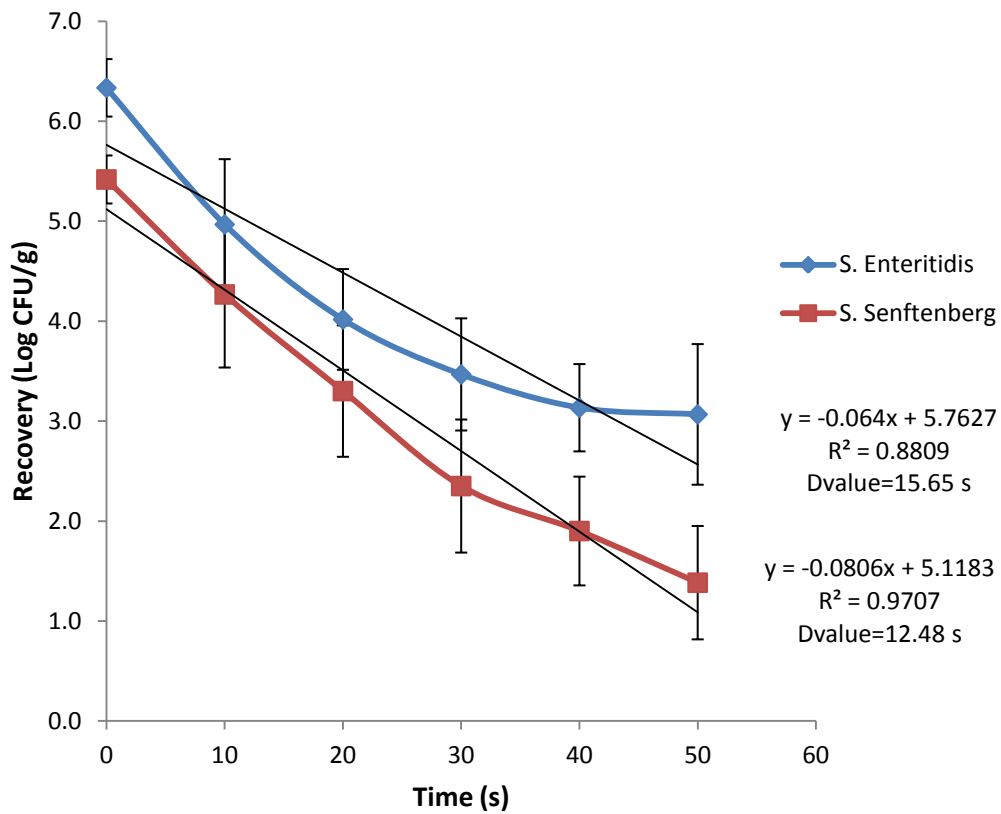
Another explanation to the large variations of D-values of *Salmonella* inoculated onto different commodities is based on the fact that microorganisms can express greater resistance to irradiation when the  $A_w$  in the matrix is low (Miller 2005). This phenomenon has been attributed to the lethal indirect effects of the free radicals of water radiolysis when interacting with microorganisms (Paterson and Loaharanu 2000, Dickson 2001). This is another possible explanation for the higher D-values found for *S. Enteritidis* and *S. Senftenberg* in the current study, compared to studies where *Salmonella* was inoculated onto high  $A_w$  matrixes (Niemira and others 2001, Palekar 2004, Prakash and others 2007).



### *Blanching*

The thermal resistance of *S. Enteritidis* PT 30 rif<sup>+</sup> and *S. Senftenberg* rif<sup>+</sup> inoculated onto Nonpareil almond surfaces was determined and compared to each other. After almonds were inoculated, their *A<sub>w</sub>* was restored to the original uninoculated almonds *A<sub>w</sub>*. Blanching thermal inactivation curves at 88 °C were constructed for each microorganism and fitted to a linear regression equation. From the inactivation curves (Fig. 10), D-values were calculated and compared between the two *Salmonella* serovars. The mean and SD of the D-value for *S. Enteritidis* PT 30 rif<sup>+</sup> was  $15.65 \pm 0.78$  s and for *S. Senftenberg* rif<sup>+</sup> was  $12.48 \pm 1.40$  s. To use blanching as the only intervention to achieve the mandatory 4-log reduction of *Salmonella*, a minimum blanching time of 63 s at 88 °C should be applied. Statistical comparison of the slopes of the linear regression curves of each serotype determined that there were no statistical differences ( $P = 0.0604$ ) between the two serotypes. Since there were no significant differences in the thermal resistance of the two strains tested, even the heat-resistant *S. Senftenberg* would be reduced by 4 log cycles when exposed to the approved almond blanching procedure for controlling *S. Enteritidis* PT 30. When Sherry and others (2004) compared the thermal resistance of 40 *Salmonella* serovars in liquid media, they found that *S. Enteritidis* PT 4 was more heat resistant than *S. Senftenberg*.

The results reported in this document are in agreement with the D-values reported by Uesugi and Harris (2005). These authors investigated the effect of blanching in almonds inoculated with *S. Enteritidis* PT 30, finding a D-value of 14.8 s. However, Uesugi and Harris (2005) did not restore the *A<sub>w</sub>*. A conservative conclusion



**Figure 10-Mean and standard deviations of the recovery of *S. Enteritidis* rif+ and *S. Senftenberg* rif+ from Nonpareil almonds blached at 88 °C at 6 time points.**

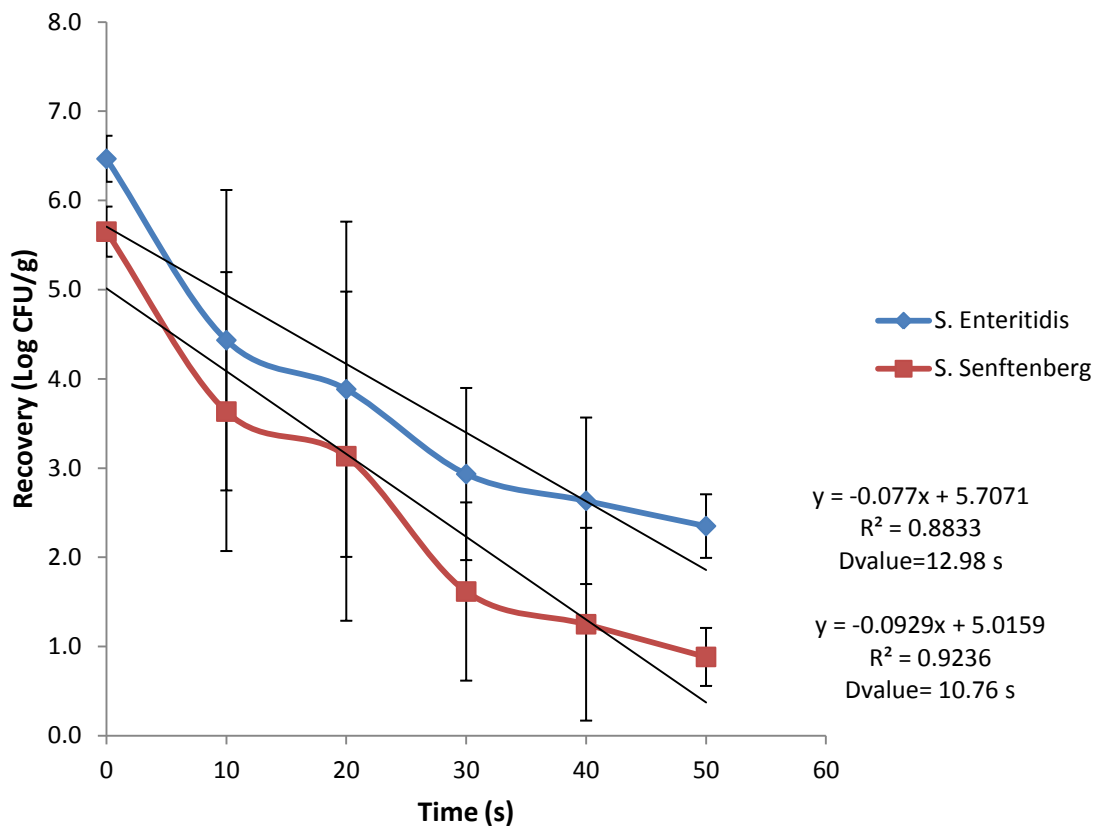
For each strain, recovery means were fitted to a linear equation from where D-values were calculated.

recommended a minimum of 2 min to ensure a 5-log reduction of *S. Enteritidis* PT 30. Based on these authors research, in 2005, the FDA issued a letter of determination acknowledging that blanching almonds at 88 °C for 2 min or longer, is a pasteurization process, and product handled under these conditions may be labeled as pasteurized.

### *Oil roasting*

The thermal resistance was determined and compared for *S. Enteritidis* rif<sup>+</sup> and *S. Senftenberg* rif<sup>+</sup> inoculated onto Nonpareil almond surfaces. After inoculation, the *A<sub>w</sub>* of inoculated almonds was restored to the original *A<sub>w</sub>* of uninoculated almonds as described in previous sections.

Thermal inactivation curves for the two *Salmonella* serovars inoculated on almonds and heated in an oil bath at 127 °C are shown in Fig. 11. The mean D-value for *S. Enteritidis* rif<sup>+</sup> was  $12.98 \pm 1.70$  s and for *S. Senftenberg* rif<sup>+</sup> was  $10.76 \pm 0.23$  s. Therefore, to use oil roasting as the only intervention to achieve a 4 log reduction of *Salmonella*, a minimum treatment of 52 s is necessary at 127 °C. Comparison of the slopes of the linear regression curves of each serotype determined that there were no statistical differences ( $P = 0.3415$ ) between the two serotypes. These results are in agreement with the D- values calculated from the values reported by Du and Harris (2005). These authors investigated the effect of oil roasting in almonds inoculated with *S. Enteritidis* PT 30, finding approximately 5-log CFU/g reduction after 90 s of oil roasting at 127 °C. However, these authors did not restore the *A<sub>w</sub>*.



**Figure 11-Mean and standard deviations of the recovery of *S. Enteritidis* rif+ and *S. Senftenberg* rif+ from Nonpareil almonds oil roasted at 127 °C at 6 time points.**

For each strain, recovery means were fitted to a linear equation from where D-values were calculated.

Based in these results, the ABC Technical Expert Review Panel recommended as a conservative measure to treat almonds for a minimum of 2 min at 127 °C. Based on these authors' research, in 2005, the FDA issued a letter of determination acknowledging that oil roasting almonds at 127 for 2 min or longer is a pasteurization process, and product handled under these conditions may be labeled as pasteurized.

**Establishment of the parameters required to achieve a 4-log reduction of a bacterial cocktail containing *S. Enteritidis* rif<sup>+</sup> and *S. Senftenberg* rif<sup>+</sup> in a commercial electron beam irradiation facility**

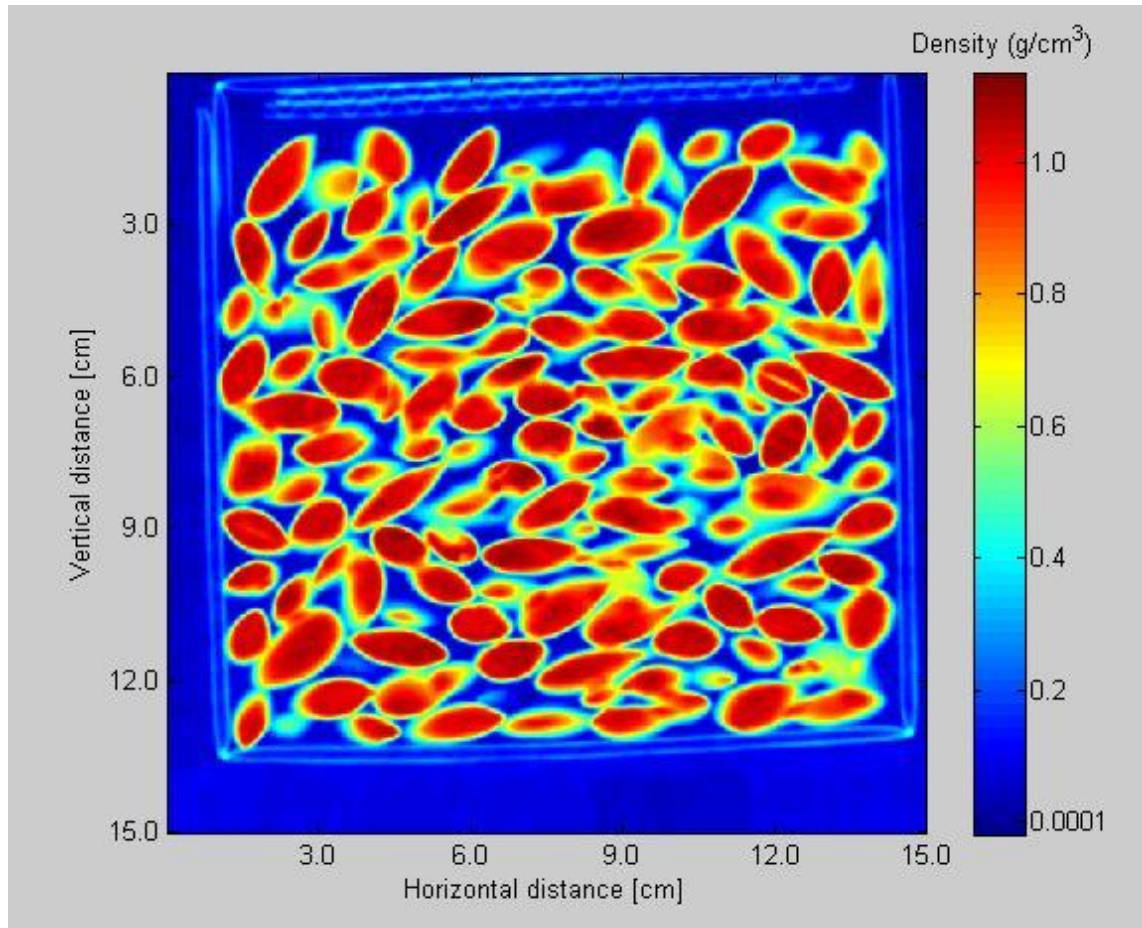
Once the irradiation D-values for *S. Enteritidis* rif<sup>+</sup> and *S. Senftenberg* rif<sup>+</sup> were accurately determined, the following step was to evaluate the feasibility of escalating the electron beam technology as an industry intervention for the almond business. The scale up of this technology to the commercial level required an assessment of the dose distribution throughout the current package configuration. Such determination was performed by MCS and compared to alanine dosimeters experimental measurements.

*Monte Carlo simulation*

Monte Carlo simulation of the interaction of 10 MeV of electrons with a 12.7 cm-height box of Nonpareil almonds was a powerful tool to observe a detailed dose distribution profile. Almonds are commercially distributed in 57 x 40 x 12.7 cm cardboard boxes, thus, for practical purposes the height of the box was maintained while the volume was reduced to 12.7 x 12.7 x 12.7 cm. A single transversal cut of the

contents of the cardboard box filled with Nonpareil almonds, as obtained by CT scan, is presented in Fig. 12. The random distribution of the almonds within the box, and the abundant air pockets inherent of almonds packed in bulk are clearly observed in this figure. Almond's density was found to be  $1.037 \text{ g/cm}^3$ , and uniform within the kernel, whereas the density of air was  $0.2 \text{ g/cm}^3$ . Figure 13 shows the dose-depth distribution of simulated single electron beam irradiation at 10 MeV. As observed in Fig. 13, the normalized entry dose at the top surface is around 0.8 with the highest dose observed around 3 cm below the surface. The high uniformity attained over the horizontal plane is explained by the squared, edge-free geometry of the box.

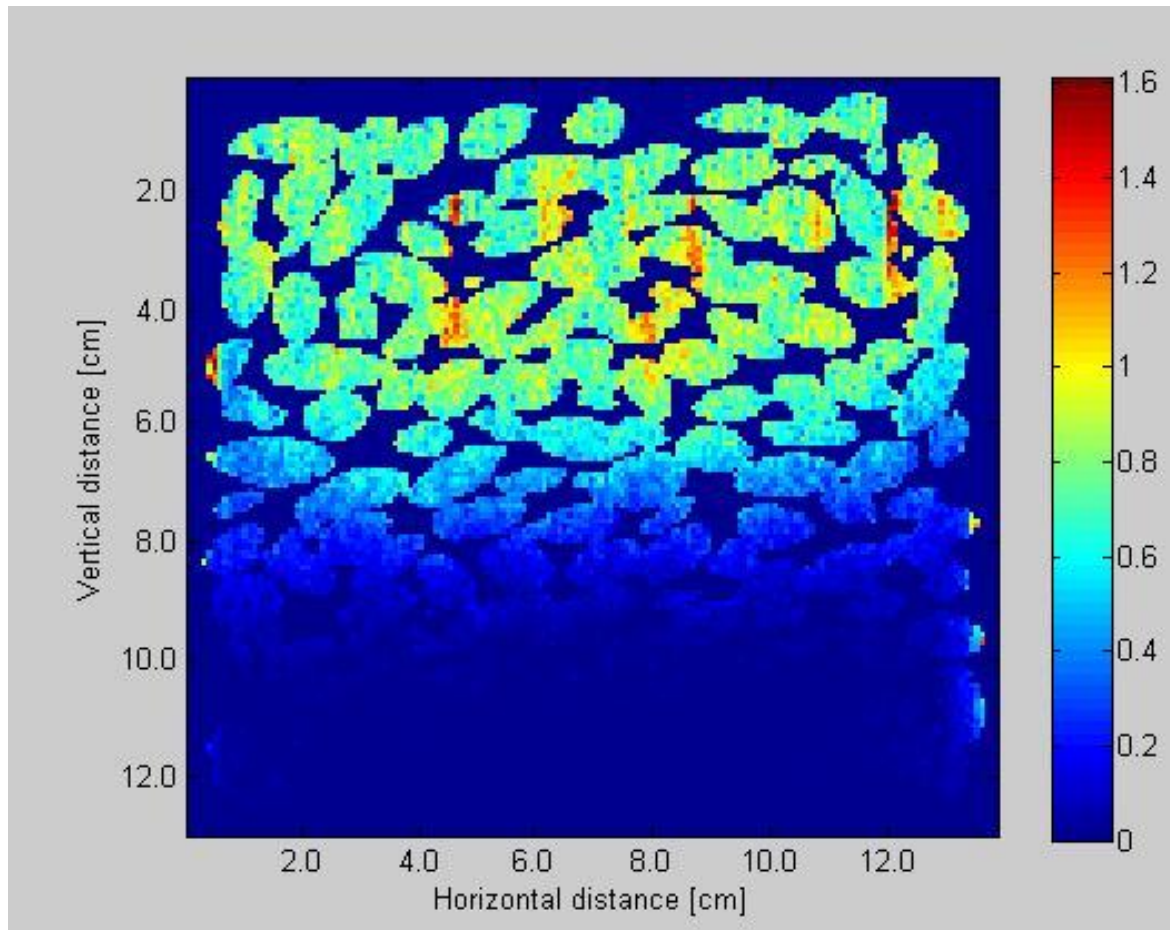
Approximately 8 cm below the surface, the energy was depleted, leaving untreated the remaining 4.7 cm of bulk almonds. Figure 14 shows the dose-depth distribution of dual electron beam irradiation at 10 MeV, according to the MCS. In this case, the normalized dose at the top and bottom surface was around 0.7. The highest dose was observed in the center of the box between 4 and 10 cm below the top surface. During a dual beam treatment, the central section of the box receives some irradiation from the top beam, which is accumulated and adds to the energy received from the bottom beam. Since the energy deposition is dependent on the depth, the DUR is an estimate of the uniformity of the dose throughout the entire depth of the product (Miller 2005). The DUR obtained from the MCS was 1.90. According to Moreno and others (2007), a DUR of 2.4 is an acceptable range in commercial applications.



**Figure 12-CT scan of Nonpareil almonds packed in bulk.**

**The scale on the right measures density ( $\text{g/cm}^3$ ).**

**Image generated with the assistance of the Department of Agricultural Engineering at TAMU.**

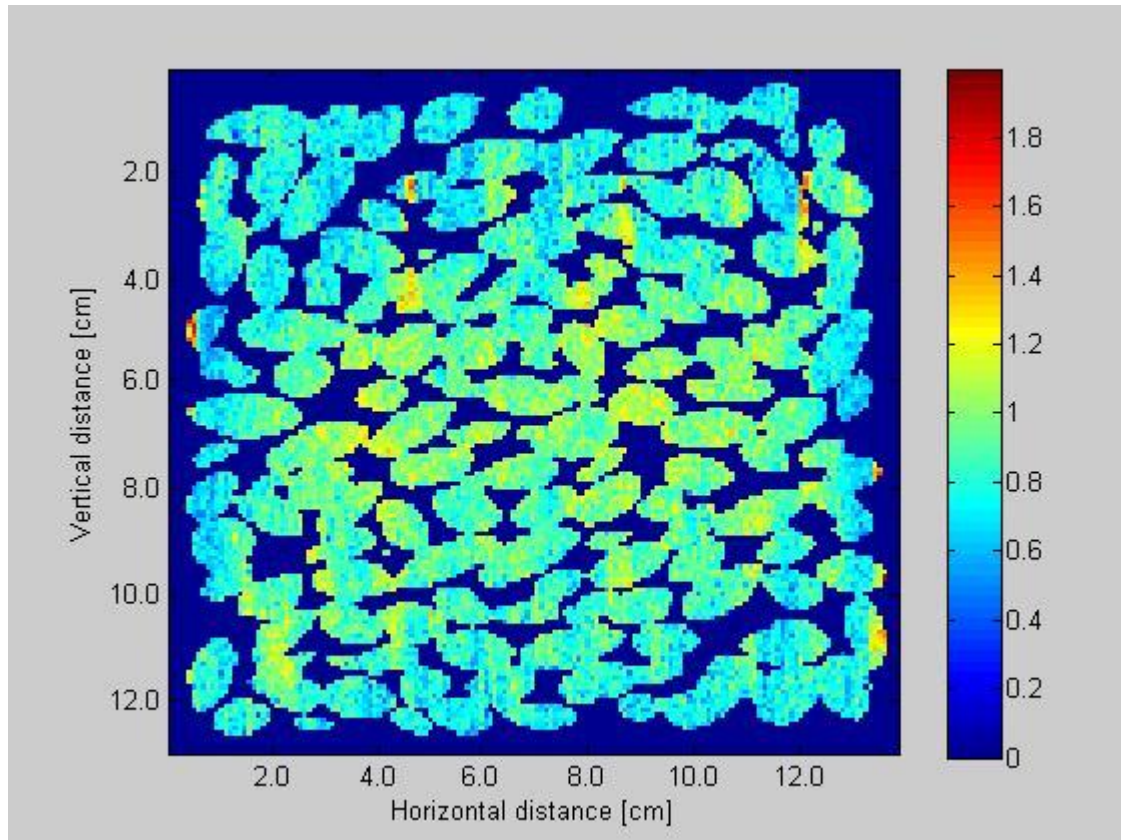


**Figure 13-Monte Carlo simulation of a box of almonds irradiated with an electron beam of 10 MeV on a single beam configuration.**

The scale on the right represents the absorbed dose at each depth (kGy).

Image generated with the assistance of the Department of Agricultural Engineering at TAMU.





**Figure 14– Monte Carlo simulation of a box of almonds irradiated with an electron beam of 10 MeV on a dual beam configuration.**

The scale on the right represents the absorbed dose at each depth (kGy).

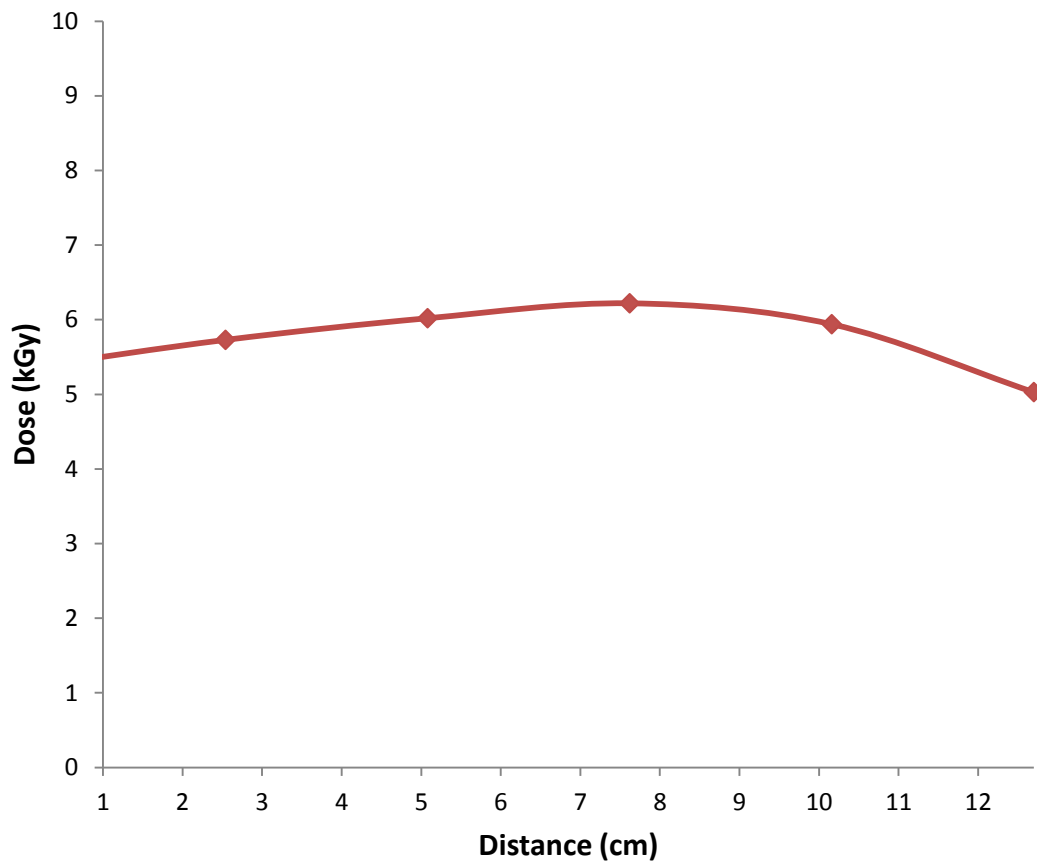
Image generated with the assistance of the Department of Agricultural Engineering at TAMU.

### *Dose-mapping using alanine dosimeters*

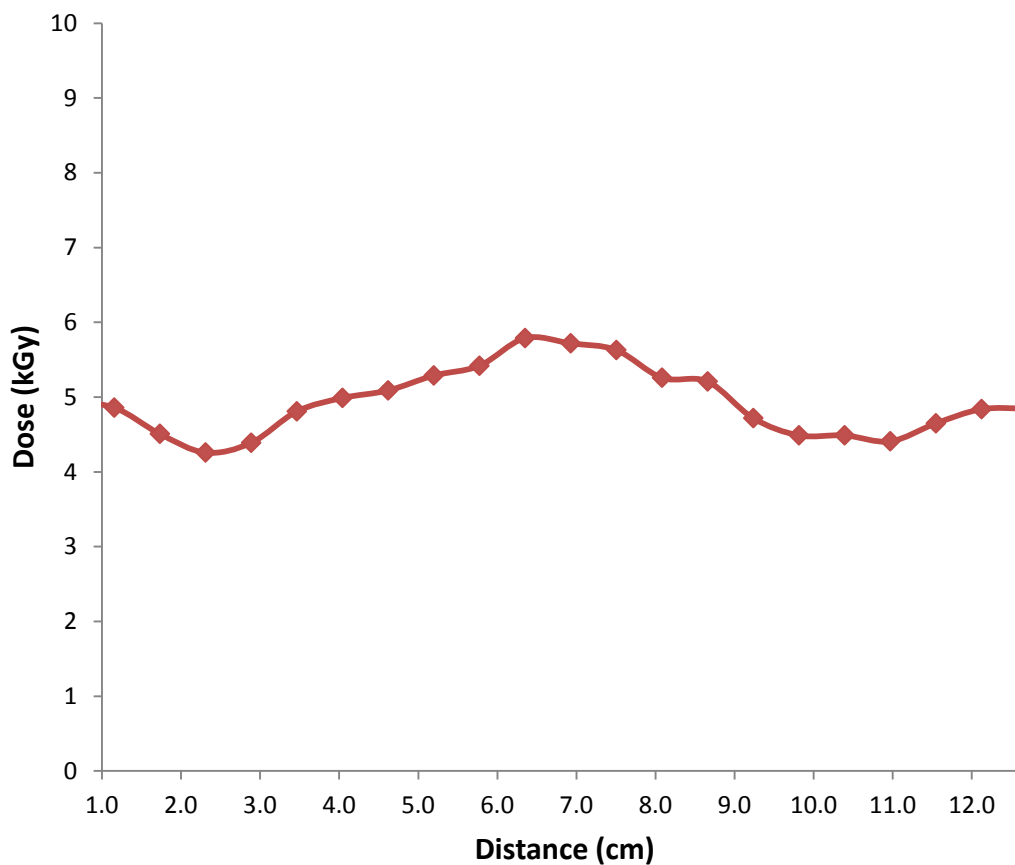
Figures 15, 16 and 17 present the dose distribution in the horizontal, vertical and diagonal plane of a 12.7 x 12.7 x 12.7 cm box filled with Nonpareil almonds. The mean absorbed dose of the 6 dosimeters placed horizontally was 5.72 kGy with a DUR of 1.23. The mean absorbed dose of the 23 vertically stacked dosimeters was 4.93 with a DUR of 1.35. Finally, the mean absorbed dose of the 12 dosimeters placed diagonally was 5.69 with a DUR of 1.23.

Figure 16 shows a typical dose-depth distribution of a dual beam process. However, the chances that almonds in bulk would randomly attain this air pocket free configuration are quite slim. Therefore, the horizontal and diagonal configurations are better representations of the possible outcome in a real scenario.

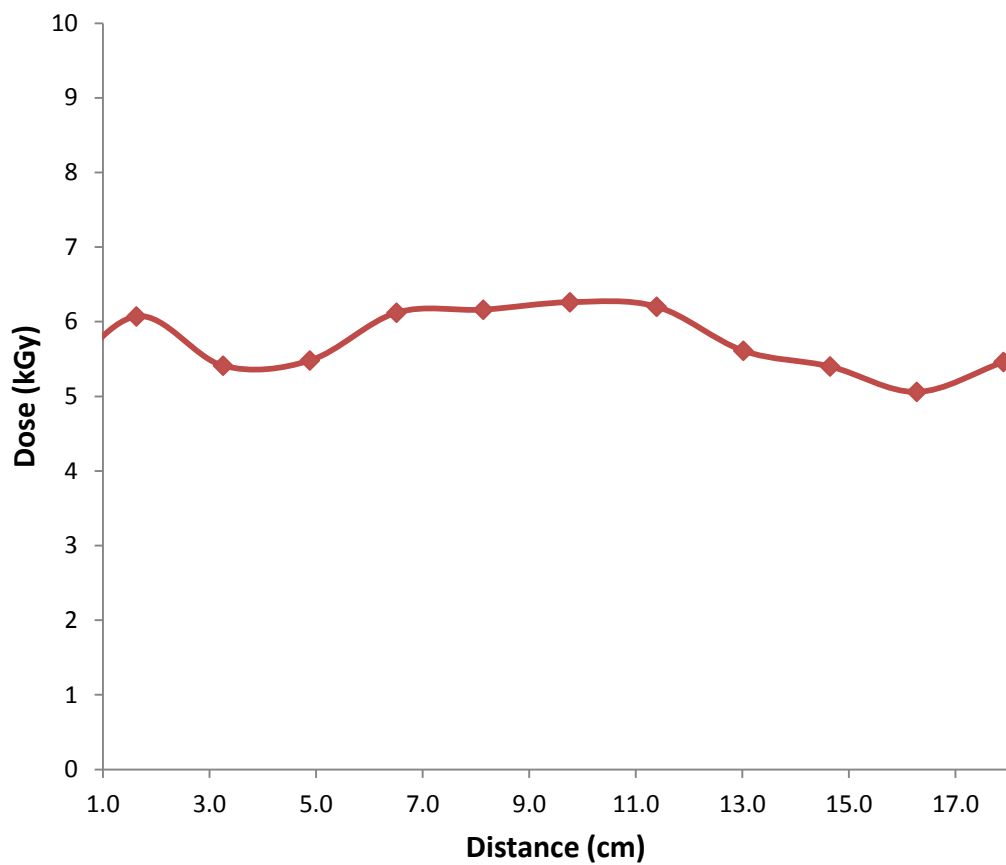
The DUR of the almonds box was 54% higher in the Monte Carlo simulation than the readings obtained experimentally. This can be explained by the volume differences between alanine pellets and voxels. Alanine dosimeters have a cylindrical shape, with a diameter of 0.48 cm, and a thickness of 0.29 cm, making a total volume of  $5.89 \times 10^{-2} \text{ cm}^3$ , which is almost 15 times bigger than the volume of a voxel. Additionally, an alanine dosimeter reading is the average of the absorbed dose over the entire pellet's volume, meaning that some regions might have absorbed quite higher doses compared to others. However, both type of determinations provided meaningful information about the dose distribution within the box of almonds, and in both cases the DUR was within the acceptable industry standards (Moreno 2007).



**Figure 15-Horizontal placement of 6 alanine dosimeters fixed into a 12.7 cm<sup>3</sup> box filled with almonds and irradiated with a linear accelerator of 10 MeV in a dual beam configuration.**



**Figure 16-Vertical assemble of 23 alanine dosimeters fixed into a 12.7 cm<sup>3</sup> box filled with almonds and irradiated with a linear accelerator of 10 MeV in a dual beam configuration.**



**Figure 17-Diagonal assemble of 12 alanine dosimeters fixed into a 12.7 cm<sup>3</sup> box, filled with almonds, and irradiated with a linear accelerator of 10 MeV in a dual beam configuration.**

## **Evaluation of the chemical and sensory quality of almonds commercially processed with electron beam irradiation, blanching, and oil roasting**

### *Sensory analysis*

The panel that participated in the consumer sensory study consisted of students, faculty, and staff from Texas A&M University. In two different days, a total of 97 consumers evaluated samples of irradiated, blanched, and oil roasted almonds. Fifteen percent of the consumers performed the test on both days. Tables 3 and 4 portray the demographic and consumption lifestyle information of the panelists. Fifty-five percent of the participants were female, 42% were in the 21 to 25 age bracket, 46% consumed nuts more than four times a month, 81% consumed almonds at least one time a month, and 26% indicated that almonds were their most frequently consumed nut.

To identify the consumer's most appreciated attributes when consuming nuts, participants were asked to rank in order of preference the following characteristics: flavor, nutrition, safety, price, organic, and freshness. These results are presented in Fig. 18. Flavor was selected as the most important attribute by more than 40% of the participants, followed by nutrition (20%). Safety was selected as the most important attribute by 7% of the participants. The least important attribute for 76% of the interviewees was organic.

The perception of consumers regarding the use of alternative methods to pasteurize almonds was assessed by asking consumers to rank, in order of acceptability, seven different technologies. The technologies presented were: fumigation, water

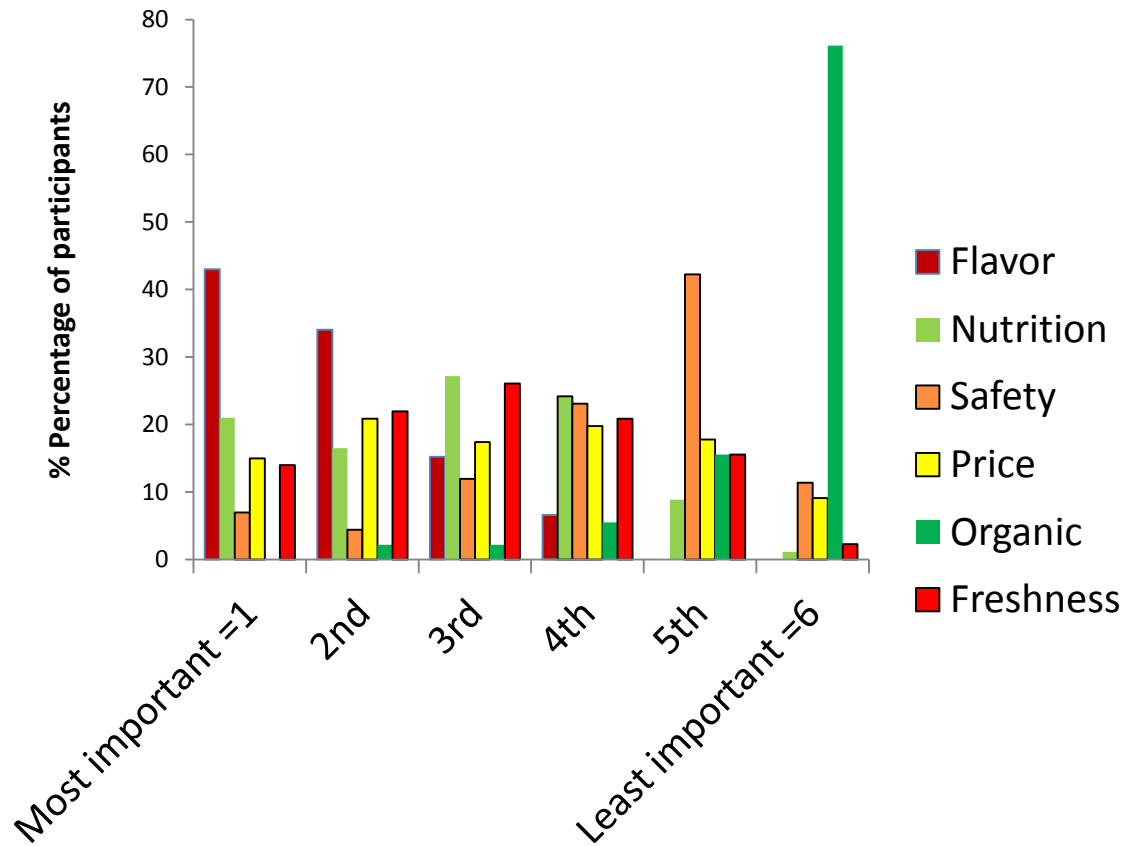
**Table 3-Demographics of consumers participated in the sensory evaluation of commercially irradiated, blanched and roasted almonds.**

<b>Gender</b>	<b>%</b>
Male	45
Female	55
<b>Age</b>	<b>%</b>
< 20	6
21-25	42
26-30	26
31-35	13
36-40	5
41-45	0
46-50	2
51-55	2
56-60	0
61-65	0
over 65	1

**Table 4-Frequency of consumption of nuts and almonds by the consumers that participated in the sensory evaluation of commercially irradiated, blanched and roasted almonds.**

<b>Consumption of nuts per month</b>	<b>%</b>
None	14
Once	19
Two times	10
Three times	11
Four times	46
Five or more times	0
<b>Consumption of almonds per month (at least)</b>	<b>%</b>
None	19
Once	81
Two times	52
Three times	42
Four times	30
Five or more times	21
<b>Most consumed nut</b>	<b>%</b>
Peanuts	43
Almonds	26
Pecans	21
Cashews	18
Pistachios	11
Walnuts	4
Hazelnuts	4



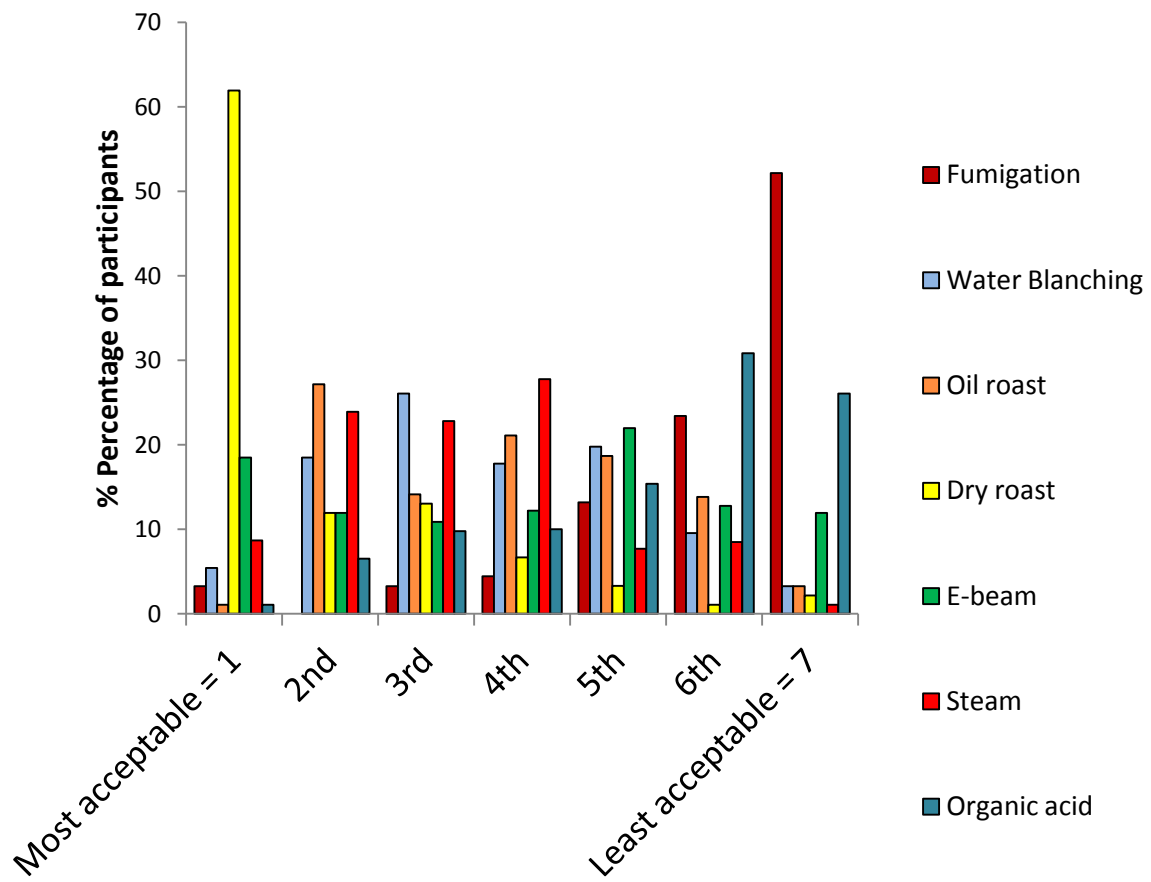


**Figure 18-Ranking of attributes according to consumer's perceptions when consuming nuts.**

blanching, oil roasting, dry roasting, electron beam pasteurization, steam, and organic acid rinses. Figure 19 shows a summary of the consumer's responses. The most acceptable technology was dry roast, selected by 60% of the participants, followed by electron beam pasteurization, preferred by 18% of the consumers. On the other side, the two least acceptable methods were fumigation and organic acid rinses, selected by 52 and 26% of the participants, correspondingly. Electron beam pasteurization was selected as the least acceptable process by only 12% of the participants.

Hedonic and intensity scales were used to grade the sensory attributes of treated almonds. A 9-point hedonic scale was used to evaluate overall like, exterior color like, interior color like, and almond flavor like. A 9-point intensity scale was used to rate texture level, bitterness level, and rancidity level. The last question in the ballot inquired about the likelihood of purchase. Hedonic and intensity ratings were anchored to like or dislike statements, (1 = dislike extremely, 9 = like extremely).

An overview of the Pearson correlations of consumer's responses is presented in Table 5. Pearson's correlation measures the strength of the linear relationship between two variables. A strong correlation implies that the two variables co-vary to a certain extent (Otto and Longnecker 2010). Interestingly, the strongest relationships in the consumer's responses were between bitterness level and rancidity level ( $r = 0.814$ ). Another remarkable relationship was observed between likelihood of purchase and almond flavor like ( $r = 0.807$ ), indicating that when it comes to purchasing almonds, the consumer's most important factor is almond flavor. This finding concurs with the previously discussed demographic data of this work, when consumers were directly



**Figure – 19 Consumers rankings according to their perceived level of acceptability of potential almond pasteurization technologies.**

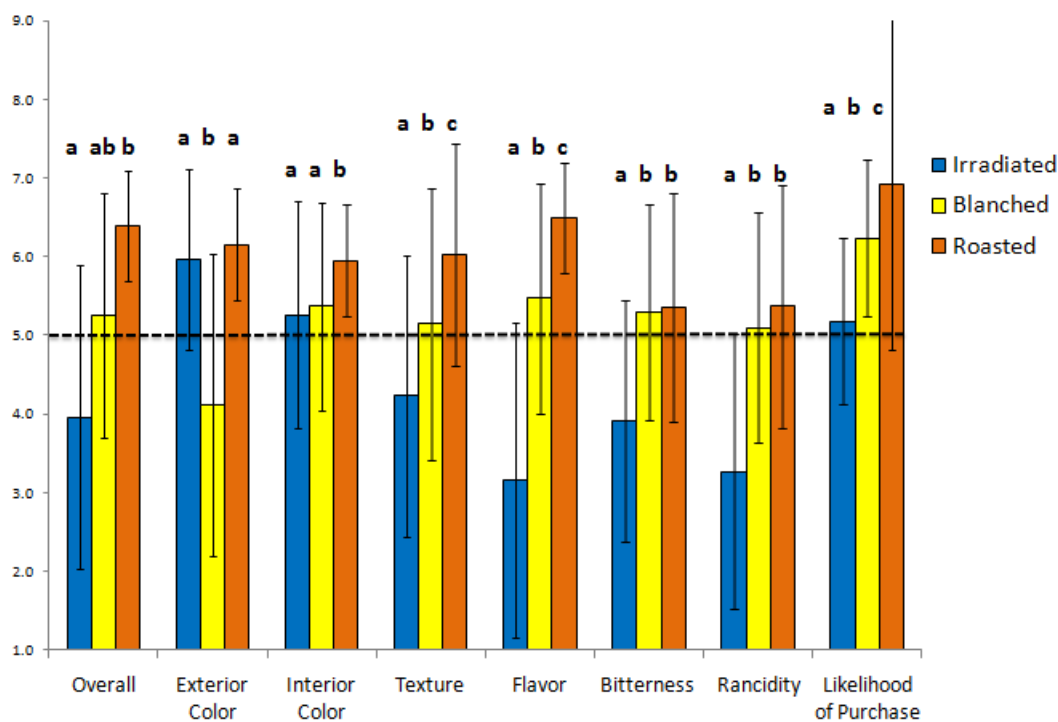
**Table 5-Correlation of attributes rated by consumers that participated in the sensory evaluation of irradiated, blanched and roasted almonds.**

	<b>Consumer Response Variable</b>							
	<b>Overall like</b>	<b>Exterior color like</b>	<b>Interior color like</b>	<b>Texture level</b>	<b>Almond flavor like</b>	<b>Bitterness level</b>	<b>Rancidity level</b>	<b>Likelihood of purchase</b>
<b>Overall like</b>	-	0.330	0.478	0.552	0.768	0.439	0.419	0.711
<b>Exterior color</b>		-	0.418	0.271	0.122	0.045	0.006	0.18
<b>Interior color</b>			-	0.465	0.449	0.318	0.325	0.365
<b>Texture level</b>				-	0.619	0.419	0.406	0.619
<b>Almond flavor</b>					-	0.591	0.615	0.807
<b>Bitterness level</b>						-	0.814	0.510
<b>Rancidity level</b>							-	0.539
<b>Likelihood of</b>								-

asked about the most important attribute when consuming nuts. More moderate relationships were observed between the consumer's perception of almond flavor like when related to texture level ( $r = 0.619$ ), rancidity level ( $r = 0.615$ ), and bitterness level ( $r = 0.59$ ) ratings.

The overall like variable was strongly related to almond flavor like ( $r = 0.768$ ), and likelihood of purchase ( $r = 0.711$ ). Still, the correlation of overall like was moderate when compared against texture level, interior color like, bitterness level, and rancidity level, respectively ( $r = 0.552, 0.478, 0.439, 0.419$ ).

Figure 20 summarizes the responses of the consumers when evaluating the irradiated, blanched and oil roasted almonds. For the overall like attribute, irradiated and oil roasted almonds were rated significantly different ( $P < 0.05$ ), but no significant differences were found between irradiated and blanched, or blanched and roasted. The mean and SD of the overall like rating for irradiated almonds was  $4.0 \pm 1.9$ , falling between the "Like slightly" and "Dislike intensely" anchors. Similar results were found by Prakash and others (2010), who performed a sensory test with a consumer panel of 58 participants. The consumers evaluated the overall likeness of almonds irradiated at 5.25 kGy, using the same 9-point scale used in the present study, and the mean overall like score of irradiated samples was 4.62. In the current study, blanched almonds received a mean and SD of the overall like grade of  $5.3 \pm 1.6$ , which placed these samples between the "Like moderately" and "Dislike slightly" anchors. The best rated samples, oil roasted almonds, received a mean and SD of overall like score of  $6.4 \pm 0.7$ , which translated into



**Figure 20-Attributes evaluated on irradiated, blanched and roasted almonds with a 9-point-hedonic and intensity scales by a consumer panel.**

9 = “Like Extremely” and 1 = “Dislike Extremely”.

The dotted line marks the neutral point of “Neither Like nor Dislike”.

Root mean square error (RMSE) for each attribute from left to right are: 0.287, 0.105, 0.541, 0.198, 0.208, 1.195, 1.274, and 0.380, respectively.

Columns with by the same letter are not significantly different ( $P > 0.05$ ).

words falls between “Like moderately” and “Like slightly”. For the exterior color like attribute of the almonds evaluated in the present work, no differences were found between irradiated and oil roasted almonds, but they were both different ( $P < 0.05$ ) to the blanched ones. However, when evaluating the interior color like, irradiated and blanched almonds ratings were not significantly different from each other, but both were significantly different ( $P < 0.05$ ) from the oil roasted almonds. This outcome was expected since the blanched almonds were skinless and oil roasting imparts a darker color.

For the texture like, and almond flavor like attributes, all the treatments were rated different from each other ( $P < 0.05$ ). Oil roasted almonds were given the highest score, followed by the blanched, and then the irradiated almonds. For bitterness level and rancidity level attributes, there were no significant differences between blanched and roasted almonds, but they both were significantly different ( $P < 0.05$ ) to the irradiated almonds.

Available literature in the irradiation of almonds topic, provide contrasting results to the present study and among each other. For instance, in sensory evaluations performed by Narvaiz and others (1992), Ribó and others (2004), and Sánchez-Bel and others (2005) there were no differences between any tested attributes at irradiation doses ranging from 0 to 7 kGy. On the other hand, Mexis and others (2009), and Prakash and others (2010) found some sensory attributes to be dose-dependent. Narvaiz and others (1992) irradiated almonds with gamma rays at 0, 1, 1.5 and 2 kGy, and stored them for 7 d and 6 mo at 5 °C. Five trained judges evaluated the almond’s external appearance,

odor, flavor, and consumer acceptability. There were no significant differences between irradiation doses regarding any attribute after 7 d or after 6 mo of storage. Ribó and others (2004) irradiated almonds with an electron accelerator at 0, 1, 3 and 5 kGy, and stored them for 4 mo at 20 °C. A panel of five trained judges evaluated sweetness, color, bitterness, texture, rancidity, and global quality attributes. The judges were not able to detect any differences between treatments regarding any of these attributes. Sánchez-Bel and others (2005), used a panel of five trained judges to evaluate the global quality, and rancidity of almonds irradiated with electron beam doses of 0, 3, 7, and 10 kGy. From the evaluations made by these judges, there were no significant differences between the control and almonds irradiated up to 7 kGy, when evaluating almond's global quality and rancidity.

Contrastingly, Mexis and others (2009) performed a consumer sensory test of almonds irradiated with gamma rays at 0, 1.5, 3, 5 and 7 kGy. Consumers evaluated these almonds for color, texture, odor and taste in a 9-point hedonic scale. Color and texture attributes were not significantly different at any irradiation dose. In contrast, taste was significantly different between all treatments. These authors concluded that the effect of the applied irradiation dose was indirectly proportional to the almonds taste. The study of Prakash and others (2010) included a descriptive sensory test with five trained judges. During the judge's training sessions, the attributes "Metallic-chemical" and "Rancid-oxidized-fatty" were indistinguishable from each other. Therefore, these two attributes were combined in a single one, "Metallic/chemical/rancid/oxidized/fatty taste". The cumulative training sessions of the judges lasted between 9 and 12 h. When



irradiated almonds were compared against a non-irradiated control, they were given lower scores in the almond flavor attribute, no change in crunchiness, and higher scores in the “Metallic/chemical/rancid/oxidized/fatty taste”.

Finally, the attribute likelihood to purchase was found to be significantly different ( $P < 0.05$ ) among the irradiated, blanched, and roasted, almonds. Consumers were more prone to purchase almonds in this order: roasted > blanched > irradiated.

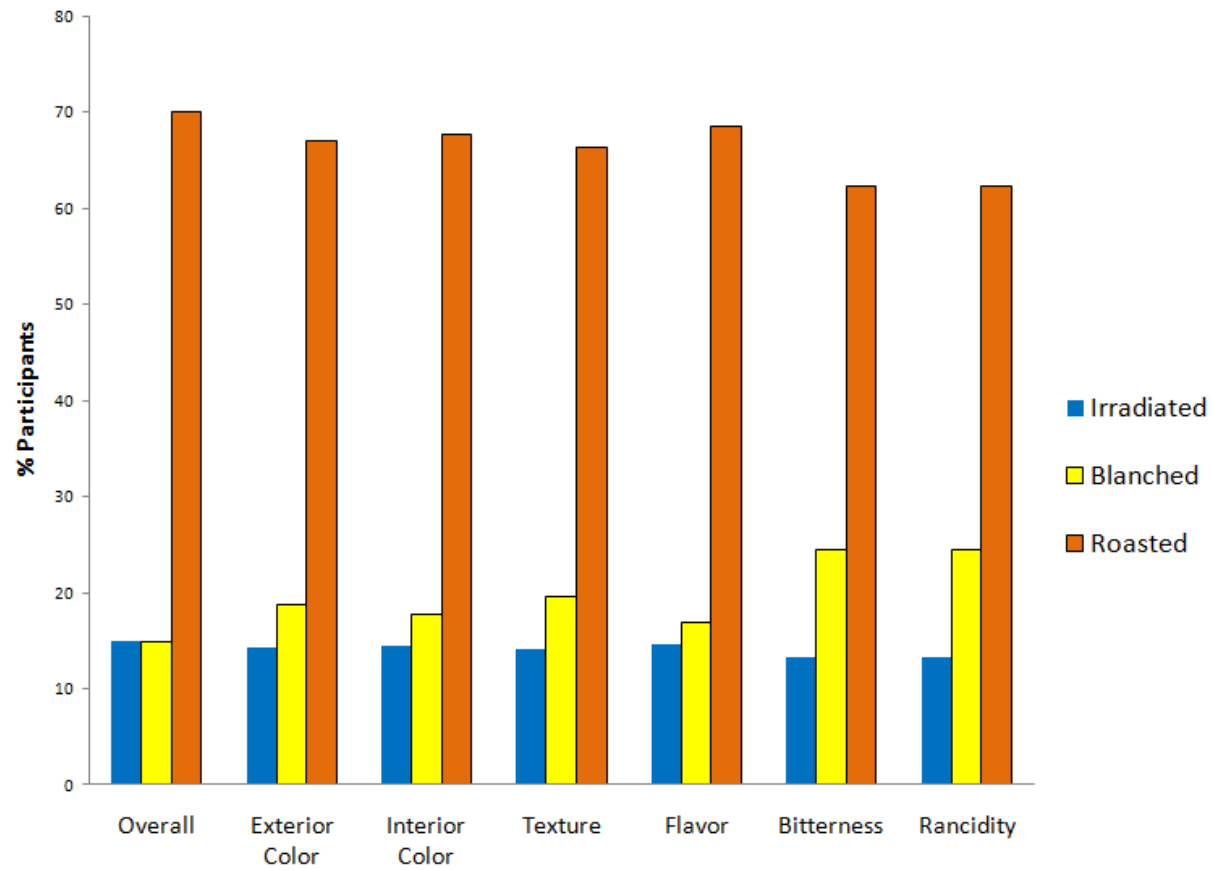
At the end of the evaluation sheet, a section for additional comments was provided to the panelist to discuss any further thoughts. The comments are summarized in Table 6. From a total of 97 panelists, 45, 47 and 20% of them had comments concerning irradiated, blanched, or roasted almonds, respectively. According to the content of comments, they were classified as “Positive”, “Indecisive”, or “Negative”. Out of all the comments that irradiated almonds received, 9 % were positive, 5% were indecisive and 80% were negative. Additionally, 7% of the comments mentioned a burnt or grilled flavor, 11% reported the perception of a stale or rancid taste, and 50% of the comments described the sample as something “strange”, “weird”, “funky”, with an “odd flavor”, “a flavor that I can’t describe”, and so on. Blanched almonds received comments that were 20% of the time positive, 24% indecisive, and 57% negative. Interestingly, 48% of the total comments concerned the lack of skin. Roasted almonds received the least amount of comments, but when comments were present, 68% of the time they were positive and 26% negative.

**Table 6-Percentage of consumer ballots that included comments, and the classification of the comments for each type of almond processing.**

Type of treatment	Consumer ballots with comments (%)	Ratings of overall comments (%)			Lack of skin*	Specific flavor detected**		
		Positive	Indecisive	Negative		Burnt	Stale/Rancid	Unidentified
<b>Irradiated</b>	45	9	5	80		7	11	50
<b>Blanched</b>	47	20	24	57	48			
<b>Oil Roasted</b>	20	68	0	26				

\*Percentage of comments related to the lack of skin in blanched almonds

\*\* Percentage of comments describing the flavor perceived in irradiated almonds



**Figure 21-Consumer preference test. Consumers were asked to selected their sample of choice between irradiated, blanched, or oil roasted almonds for each attribute.**

In the preference test, consumers were asked to select their product of choice for each attribute. When consumers were asked about the overall like preference, 70% selected roasted almonds, 15% affirmed they preferred irradiated almonds and, 15% chose blanched almonds (Figure 21). For exterior color like, interior color, texture level, and almond flavor like attributes, about 5 % more consumers preferred blanched against irradiated almonds. However, for bitterness level and rancidity level, about 11% more consumers preferred blanched against irradiated.

There are three major findings in the work described in the current section. First, the attribute almond flavor was confirmed to be the most important attribute that drives consumer's preference. This finding was confirmed by Pearson correlation analysis and by direct inquires to consumers. Secondly, it is paramount to address the contradictory results found in various sensory studies of irradiated almonds. Differences in the detection of bitterness and rancidity characters among different studies may be attributed to the unmeasured lipid oxidation state of the almonds before irradiation. In other words, if raw almonds were already at an advance stage of lipid oxidation, the irradiation treatment may have had little contribution to the overall quality of the irradiated almonds. Finally, in the present study, a considerable number of consumers commented about the perception of an undefined flavor in irradiated almonds. This suggests that the overall flavor of irradiated almonds is affected by the presence of something more than the commonly known lipid oxidation breakdown products. This finding is supported by the study of Prakash and others (2010), where trained judges were unable to separate the "Metallic-chemical" from the "Rancid-oxidized-fatty" taste.

### *Oxidation of almond's lipid fraction*

Foods that contain high concentrations of unsaturated lipids are particularly susceptible to lipid oxidation. Lipid oxidation is one of the major forms of spoilage in foods, because it leads to the formation of off-flavors which have great impact on the acceptability of the product. Lipid oxidation is a complex process involving numerous reactions that give rise to a variety of chemical changes in lipids. In general, in a reaction that involves unsaturated fatty acids and O<sub>2</sub>, the primary products are peroxides and conjugated dienes, which further react to form secondary products such as aldehydes, ketones, alcohols and, hydrocarbons (Fenemma 2000, and Mexis and others 2009). Due to the high fat content of almonds, almost 50% of their dry weight, and their unsaturated fatty acid profile (USDA 2010), the most likely form of quality deterioration is the oxidation of the unsaturated fatty acids.

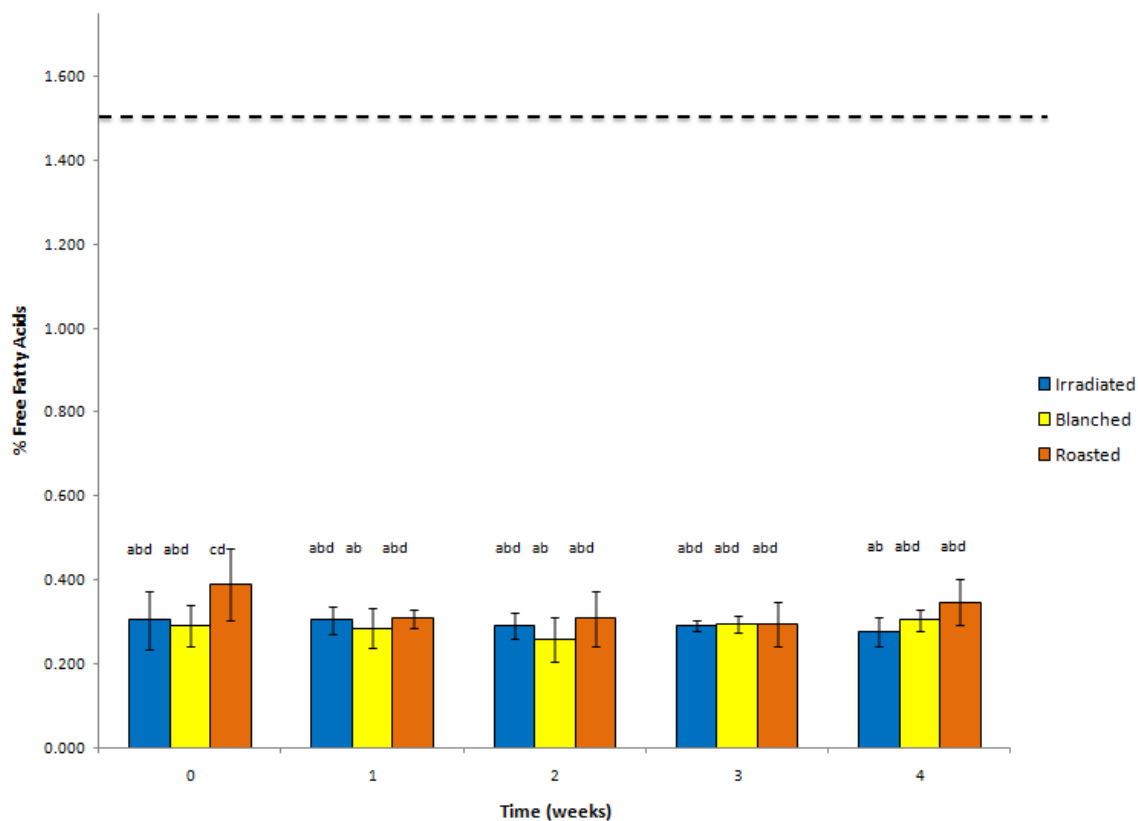
In the current study, quantitative and qualitative determinations of fat oxidation were performed. To quantitatively assess the quality deterioration of commercially irradiated, blanched, and oil roasted almonds, fat oxidation was chemically measured by testing the %FFA, PV, and TBARs. To accelerate the degradation reactions, and simulate one-half of the almond's commercial shelf life, irradiated, blanched, and roasted almonds were subjected to an ASLT, at 50 °C and 20% RH for 4 weeks. Throughout this 4-week period, %FFA, PV, and TBARs assays were performed weekly. Additionally, gas chromatography/mass spectrometry analysis was performed with the purpose of further investigating the effect of irradiation on almonds. To qualitatively

assess the odor impact of the volatiles present in irradiated almonds, aroma descriptors and their odor intensity were gathered and used to construct an aromagram.

#### *Percentage of free fatty acids*

Figure 22 presents the means and standard deviations of %FFA of irradiated, blanched and roasted almonds across time. According to the ABC (2010c) an acceptable standard for %FFA in almonds was  $< 1.5\%$ . However, more strict specifications were established by Paramount Farm's, which %FFA specification for good quality almonds is  $< 1.0\%$  (Buransompob and others 2003). In this ASLT, the storage time was equivalent to 1 year at  $25\text{ }^{\circ}\text{C}$ , or half the commercial almond's shelf life. During this time, none of the samples had more than  $0.6\%$ FFA at any given time. Neither irradiated, blanched or oil roasted samples had significantly different %FFA values, among each other, or across time ( $P > 0.05$ ).

Similar results were observed in several studies. For instance, Narvaiz and others (1992) determined the %FFA of almonds treated with gamma irradiation at doses of 0, 1, 1.5 and 2 kGy. Almonds that were stored at  $5\text{ }^{\circ}\text{C}$  for 160 d had %FFA values between 0.20 and 0.26. Concurrent with our study findings, the %FFA of gamma-irradiated almonds appears to be static across an extended period of time, in Narvaiz and others (1992) case, for more than 5 mo.



**Figure 22-Means and standard deviations of % free fatty acids of almonds commercially irradiated, blanched or oil roasted, and stored at 50°C and 20% of RH for 4 weeks.**

The dotted line represents the industry standard (1.5 %).

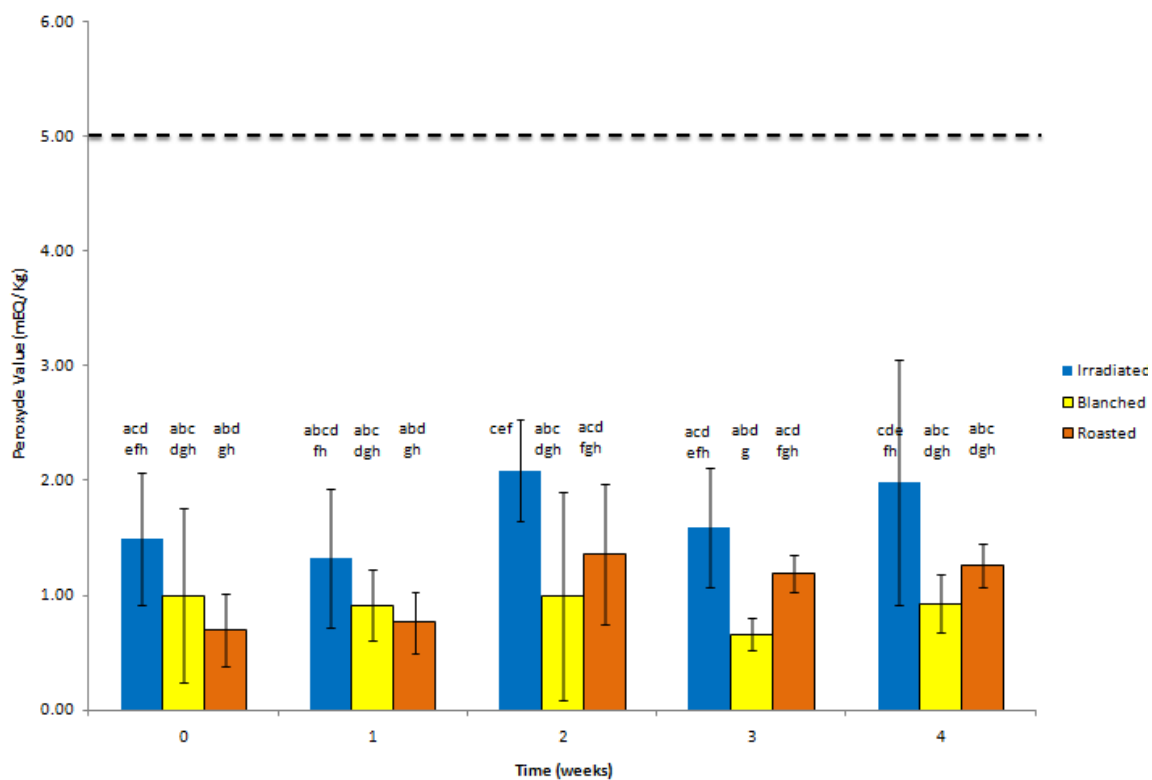
Columns with by the same letter are not significantly different ( $P > 0.05$ ).

### *Peroxide value*

Figure 23 presents the means and standard deviations of PV of irradiated, blanched, and roasted almonds across time. Peroxides are one of the primary reaction products formed in the initial stages of oxidation, and therefore provide an indication of the progress of lipid oxidation (Wrolstad and others 2005). According to the ABC (2010c) specifications, good quality almonds should have PV values of  $< 5$  meq/Kg, while Paramount Farm's PV specification for good quality almonds is  $< 2.0$  meq/Kg (Buransompob and others 2003). Based on the previously mentioned standards, good quality almonds should have PV of  $< 2.5$  meq/Kg or  $< 1.0$  meq/Kg, at one half of their shelf life. In the present study, blanched almonds had mean PV below the 1.0 meq/Kg target every week except at time 0, roasted almonds were above the 1.0 meq/Kg mark at weeks 2, 3 and 4, and finally, irradiated almonds were always above the 1.0 meq/Kg specification, from week 0 to week 4. However, neither irradiated, blanched or roasted samples had significantly different PV, among each other, or across time ( $P > 0.05$ ).

Several studies have reported similar results, for example, Sánchez-Bel and others (2005) published that PV of almonds irradiated at doses up to 7 kGy were not significantly different from the non-treated samples right after the application of the treatment, or after 5 mo of storage at 20°C. Ribó and others (2004) described that PV of almonds irradiated with an electron accelerator at 0, 1, 3 and 5 kGy were not significantly different from each other after storage for up to 4 mo at 20 °C. Mexis and others (2009) treated almonds using gamma rays with doses ranging from 0 to 7 kGy. After 28 d of storage, the mean PV of almonds treated at 0, 1.5, 3, 5 and 7 kGy, were,





**Figure 23-Means and standard deviations of peroxide values (meq/Kg) of almonds commercially treated by irradiation, blanching or oil roasting, stored for 4 weeks at 50 °C and 20% of RH.**

The dotted line represents the industry standard (5 meq/Kg).

Columns with by the same letter are not significantly different ( $P > 0.05$ ).

0.26, 1.8, 2.46 and 2.74 meq/Kg, respectively.

Other studies have observed higher PV in irradiated almonds. For example, Narvaiz and others (1992) treated almonds with 2 kGy of gamma irradiation. The irradiated almonds were stored for 3 d at 5 °C and their PV were around 5 meq/Kg. In the same study, irradiated almonds that were stored for 160 d at 5 °C reached PV of 12 meq/Kg. In a similar study by Uthman and others (1998) almonds were treated with 6 kGy of gamma irradiation, dry roasting process, or a no-treatment control. Immediately after processing, PV were 4.8, 10.5 and 2.5, correspondingly. After 16 wk of storage at 20 °C, PV increased to 17, 20, and 18.5, respectively. García-Pascual and others (2003) compared the PV of Nonpareil raw and roasted almonds throughout a period of 4 mo at 36 °C. PV increased with time in both types of almonds, but the roasted ones increased at a higher rate. Raw almonds started with a PV of 5.8 meq/Kg, and at the end of the 4 mo reached 19.5 meq/Kg. On the other hand, roasted almonds initial PV was 4.7 meq/Kg, and increased to 46.5 meq/Kg at the end of the 4 wk.

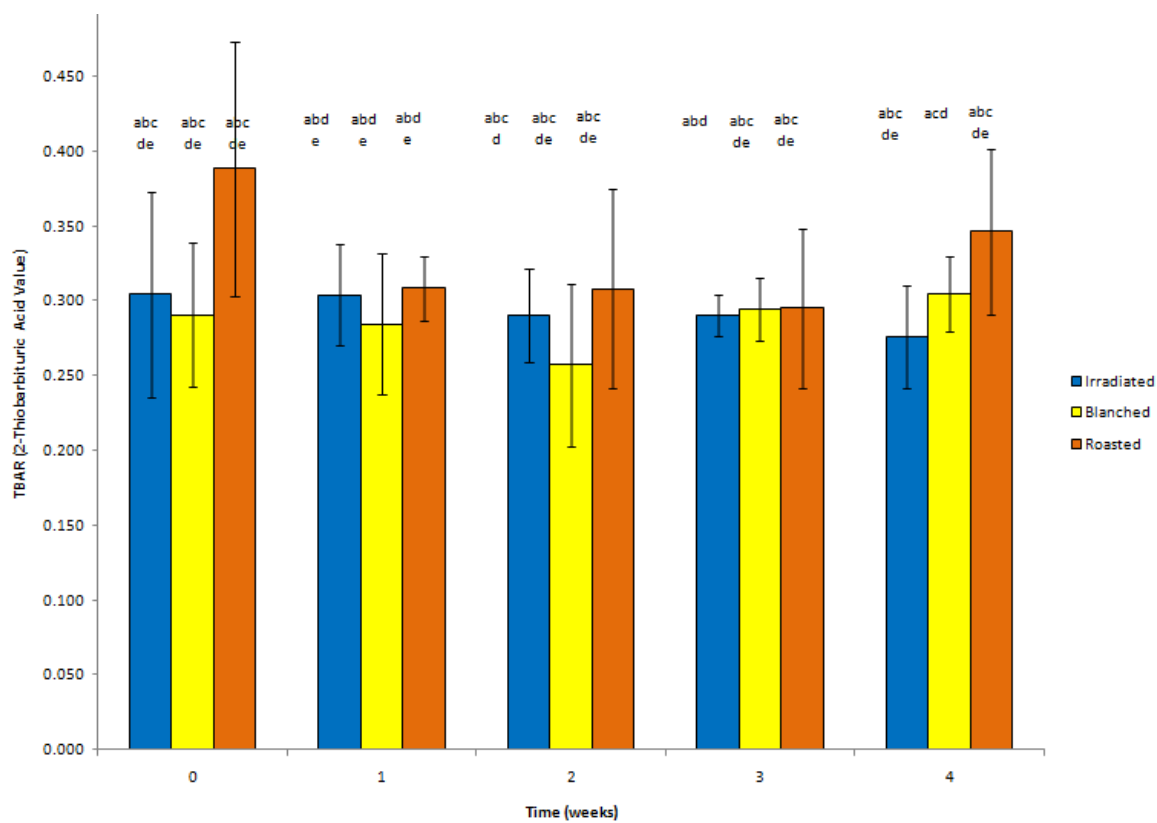
The aforementioned discrepancies in the different studies may be explained by the mechanism behind the PV test. Peroxides are one of the primary products in lipid oxidation, and they continue to breakdown in latter stages of lipid oxidation. Therefore, a low value of PV may represent either the initial or the final stage of the oxidation reaction. Additionally, the initial state of the almond's lipids among different studies is unlikely to be the same, making comparisons between experiments difficult to concur.

### *2-thiobarbituric acid reactive substances*

Figure 24 presents the means and standard deviations of TBARs of irradiated, blanched and oil roasted almonds across time. TBARs test is one of the most widely used tests for determining the extent of lipid oxidation in the meat industry. However, it is not a commonly used test in the almond industry, and therefore there is not a standard or recommended value related to good quality. Nonetheless, a measurement of the progression of the almond's lipid oxidation was necessary to fully capture the almond's quality decay across time. Since the TBARs test measures the concentration of aldehydes, one of the secondary products in lipid oxidation, this was the test of choice.

In the present study, neither irradiated, blanched or oil roasted samples had significantly different ( $P > 0.05$ ) TBARs among each other, or across time. TBARs ranged between 0.2 and almost 0.5. Similar results were observed by the only other study that has tested TBARs in irradiated almonds. Uthman and others (1998), reported that the TBARs of almonds treated with 6 kGy of gamma irradiation, dry roasting and raw control were 0.68, 0.41 and 0.28, respectively. Within the same study, a second TBARs test was performed after the irradiated almonds were stored for 16 wk at 24 °C. The mean values for the irradiated, roasted, and control almonds were 0.7, 0.62 and 0.36, respectively.

Analytical assessment of almond's oil quality is a complex task. The currently used methods determine transient chemicals, which do not necessarily correlate with sensory attributes. Additionally, when determining the overall quality of irradiated almonds, all the available literature focuses on the deterioration of the lipid fraction.



**Figure 24-Means and standard deviations of 2-thiobarbituric acid reactive substances of almonds commercially treated by irradiation, blanching or oil roasting, stored for 4 weeks at 50 °C and RH of 20%.**

Columns with by the same letter are not significantly different ( $P > 0.05$ ).

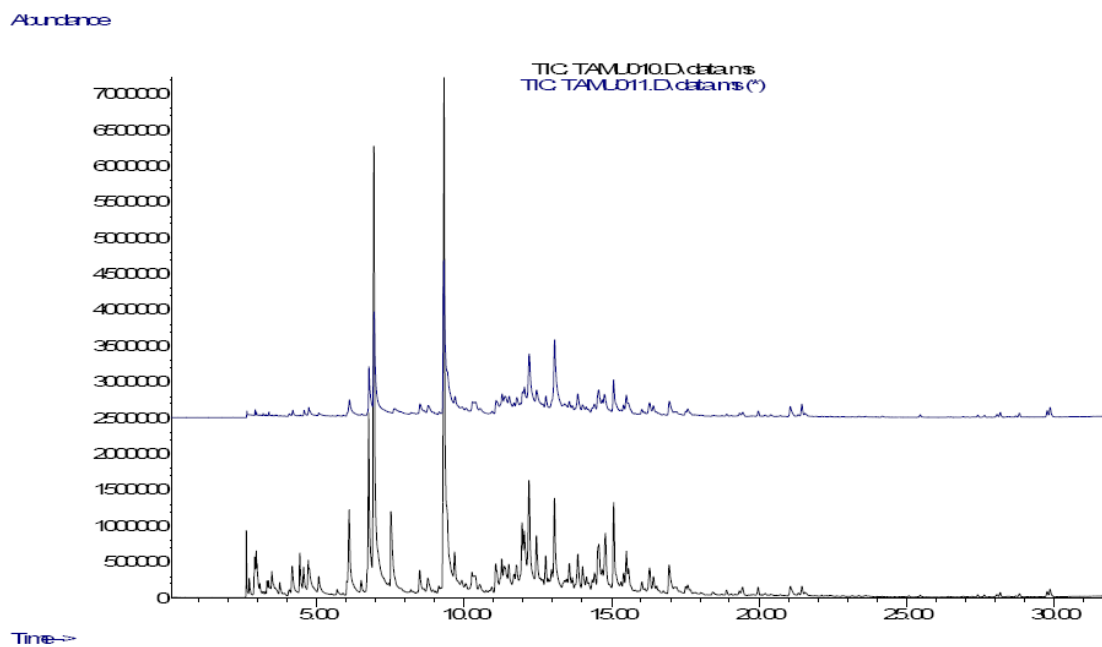
However, as it has been previously mentioned in this work, consumers have noted the presence of unknown chemicals that are not typically recognized as fat oxidation.

*Gas chromatography and mass spectrometry profile of raw and irradiated almonds*

A comprehensive aroma/flavor profile of raw and irradiated almonds using GC-MS was performed by a third party laboratory. Raw and irradiated almond samples were stored in sealed containers for 21 h, and volatile compounds were collected from the headspace. A large number of volatile compounds were detected in the headspace of each sample. The combination of all the aroma compounds constructs the almond's flavor profile.

In Fig. 25, the blue line represents the volatile compounds captured from the raw almonds, whereas, the black line represents the volatile compounds detected from the irradiated almonds. As illustrated in the chromatogram of Fig. 25, the overall volatile composition is very similar for both samples, although, the concentration of volatiles was higher in the irradiated samples. Lower concentrations of fat oxidation volatiles were present in the raw almond chromatogram indicating that the raw almonds had already started an oxidation process as well.

To observe in greater detail the differences between the raw and irradiated samples, the chromatogram presented in Fig. 25 was divided into smaller retention time windows.

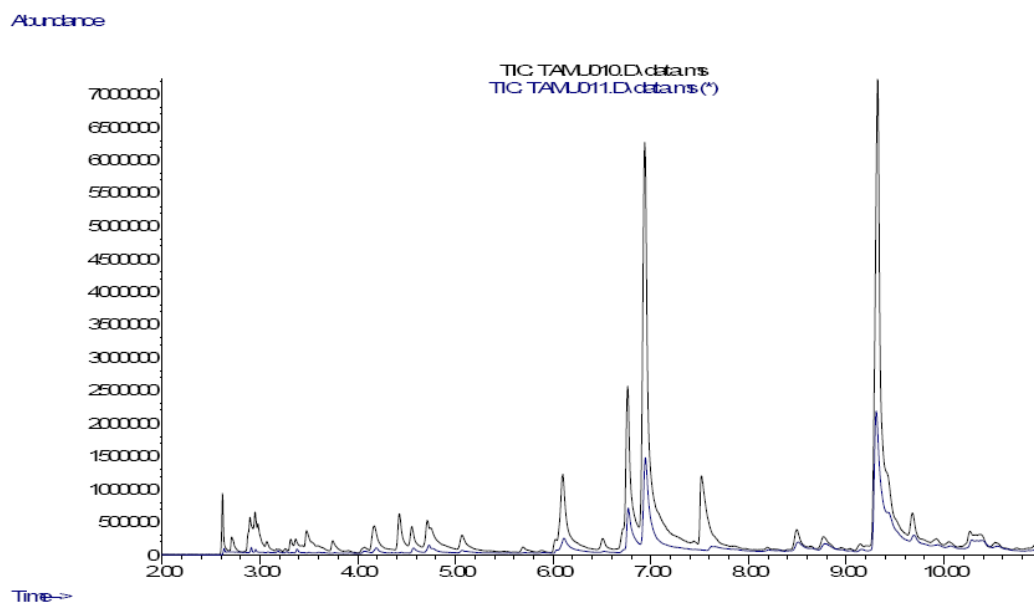


**Figure 25-GC/MS Chromatograms of the raw (blue line) and irradiated almonds (black line).**

The resulting chromatograms are presented in Figs. 26 to 28. Based on the results presented in Figs. 25 to 28, irradiation appears to have an effect in the amount and concentration of volatiles in the headspace of the samples. According to Dupuy and others (1973), and Dupuy (1976), oils that have been graded with high flavor scores show a fewer number of volatiles and lower abundance, than oils rated as low flavor quality.

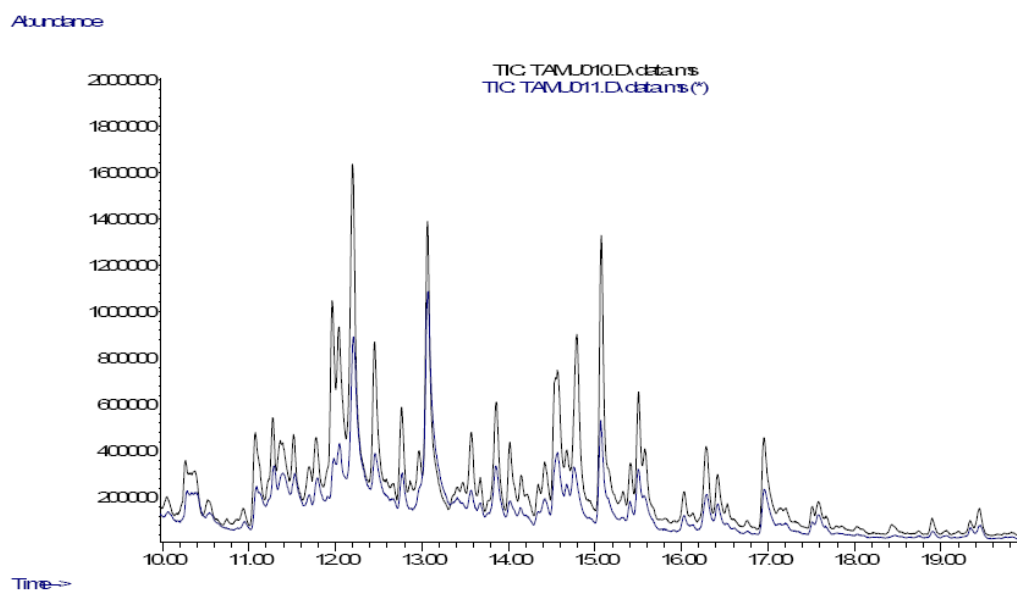
Most of the major volatiles found in each chromatographic section were identified, and they are listed in Table 7. This table presents a compilation of the volatiles obtained from the raw and the irradiated almonds, and their match with the work of Jo and Ahn (2000), and Lee and Ahn (2003). In the current study, 39 volatiles were identified in irradiated almonds, 21% of these volatiles were also reported as OFA and/or LFA radiolysis products in the work of Lee and Ahn (2003).

According to the USDA National Nutrient Database for Standard Reference (2010), raw almonds lipid profile is composed of 32% of monosaturated fatty acids (MUFAs), mainly oleic acid (18:1), and 12% of polyunsaturated fatty acids (PUFAs), mainly linoleic acid (18:2) (USDA 2010). Therefore, in the determination of raw and irradiated almond's volatiles it was expected to find the corresponding radiolysis residues of 18:1 and 18:2.

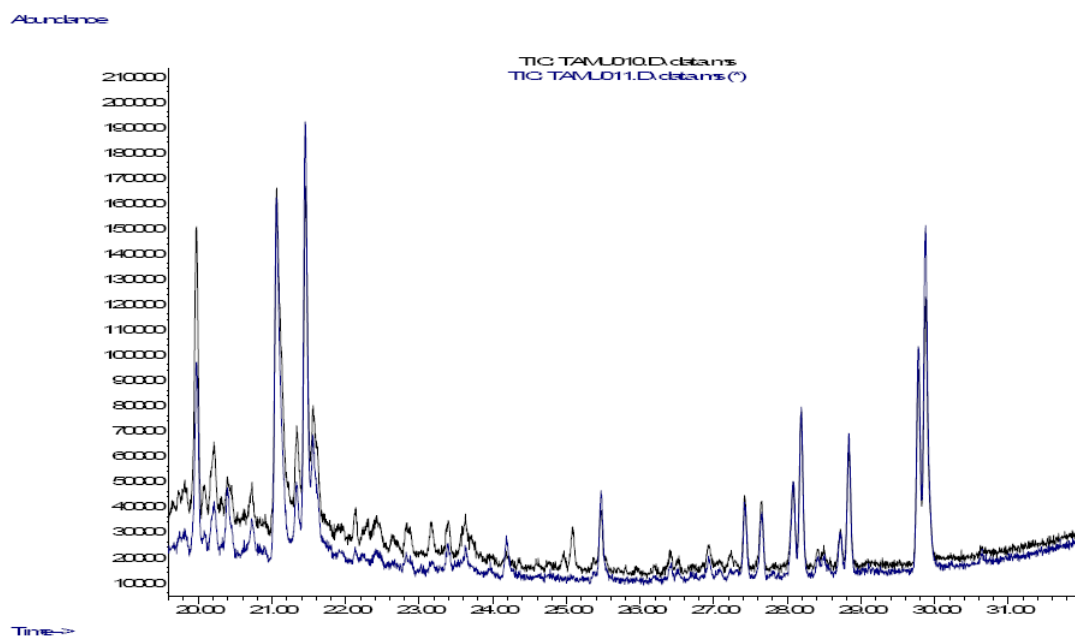


**Figure 26-GC/MS Chromatograms of the raw (blue line) and irradiated almonds (black line) for the retention time window of 2-12 min.**





**Figure 27-GC/MS Chromatograms of the raw (blue line) and irradiated almonds (black line) for the retention time window of 10-20 min.**



**Figure 28-GC/MS Chromatograms of the raw (blue line) and irradiated almonds (black line) for the retention time window of 19.6-32 min.**

**Table 7. Volatiles identified in irradiated samples by GC/MS and matched to previous**

<b>reports</b>				
Compound	Retention time	Present in	Present in	Possible by-product of almond
2-methyl propene	2.72	No	No	Yes, Methionine
1-hexene	3.32	No	Yes ↑	
Hexane	3.36	Yes ↑	Yes↑	
Methyl ethyl ketone	3.48	No	No	
2-Butanol	3.75	No	No	
3-Methyl butanal	4.07	No	No	Oil emulsion with myofibrillar
Benzene	4.17	Yes↓	No	Yes, Phenylalanine
1-Heptene	4.43	Yes ↑	Yes ↑	
Heptane	4.56	Yes ↑	Yes ↑	
Pentanal	4.71	Yes↓	Yes↓	
3-methyl-1-butanol	6.03	No	No	Oil emulsion with Leucine
Toluene	6.1	Yes↓	No	
Octene	6.51	No	No	
n-Pentanol	6.77	No	No	
n-Hexanal	6.95	Yes↓	Yes↓	
Acetic acid	7.52	No	No	
Xylene	8.77	No	No	
n-Hexanol	9.33	No	No	
n-Heptanal	9.68	No	No	
Ethyl toluene	11.27	No	No	
Unknown (ethyl toluene)	11.4	No	No	Yes, Tyrosine
1,3,5-Trimethyl benzene	11.52	No	No	Yes, Phenylalanine
n-Heptanol	11.97	No	No	
2-Amyl furan	12.04	No	No	
Decane	12.2	No	No	
n-Octanal	12.45	No	No	
d-Limonene	13.06	No	No	
2-Octenal	14.02	No	No	
Hexanoic acid	14.79	No	No	
n-Nonanal	15.07	No	No	
1,2,3,4-Tetramethyl	15.5	No	No	
Phenol	16.42	No	No	
1-Nonanol	16.95	No	No	
n-Decanal	17.52	No	No	
Naphtalene	17.59	No	No	
p-cresol	17.94	No	No	Yes, Tyrosine
2,2,4,4,6,8,8-Heptamethyl	19.96	No	No	
Isobutyl isobutyrate	21.07	No	No	
n-Butyl butyrate	21.47	No	No	

OFA: oleic fatty acid; LFA: linoleic fatty acid

\*According to the work of Lee and Ahn (2003). \*\* According to the work of Jo and Ahn (2000).

Lee and Ahn (2003) determined the volatiles produced after individually irradiating oleic (OFA) and linoleic (LFA) fatty acids with a dose of 5 kGy.

Comparisons between a control and the irradiated fatty acids, showed that the abundance of some volatiles increase, while others decrease after irradiation. For instance, in OFA, propanal, 1-pentene, pentane, 1-hexene, hexane, 1-heptene, heptane, 1-octene, and octane, increased in abundance. However, some of the ones that decreased in concentration were pentanal, hexanal, toluene and methyl cyclopentane. In the case of LFA some of the volatiles that increased in abundance were: butane, 1-pentene, 2-pentene, pentene, 3-methylene pentene, 2-hexene, hexane, 1-heptene, heptane, 1-heptyne, 1-octene, octane, 1,2-dimethyl cyclopropane, and 1-methyl cyclopentene.

Some of the volatiles that decreased were: 1,1-oxybis ethane, 2,3-dimethyl butane, butanal, 3-methyl pentane, pentanal, 1-hexene, 3-hexane, hexanal, 2-heptenal, 2-octene, 2-propyl furan, 2-buthyl furan, benzene, ethyl benzene, 1,3-dimethyl benzene, 1,4-dimethyl benzene, methyl cyclopentane, and cyclohexane.

Although, fat hydroperoxides are usually tasteless, the by-products of their decomposition have an important impact on flavor, some of them have sensory thresholds of less than 1 ppm. The breakdown products of hydroperoxides include alcohols, carbonyl compounds, acids, and the production of esters from alcohols, acids, and aldehydes (Frankel 1982). The initiated breakdown cascade of the almond's hydroperoxides was evident by the presence of alcohols and their hydroperoxide precursor. The alcohols observed in the almond's oil samples were: 2-butanol, 3-methyl-1-butanol, n-pentanol, n-hexanol, n-heptanol, and 1-nonanol. According to Frankel

(1982), photoinduced oxidation produces 9- and 10-hydroperoxides in linoleic acid. Early studies have indicated that the decomposition of 11-hydroperoxide leads to the formation of 2-octenal, which was found in the chromatogram of the almonds tested in the current study. Other reports have shown evidence that 2-octenal could also originate from the 9-hydroperoxides produced in the breakdown of linoleic acid, which is also present at lower concentrations in almonds (USDA 2010).

The almond's fatty acids of the current work presented some degree of secondary oxidation, confirmed by the aldehydes identified in the GC/MS analysis. These aldehydes were: pentanal, n-hexanal, n-heptanal, n-octanal, n-nonanal, and n-decanal. Additionally, the lipid fraction of almonds also includes a small portion of linolenic acid (USDA 2010), which oxidizes to produce volatiles that include acetaldehyde, propanal, 2-pentanal, and methyl ethyl ketone (Frankel 1982). Propanal, pentanal, and methyl ethyl ketone were found in the irradiated almonds of the current study.

As Table 7 shows, a great number of the volatiles in the almond samples were compounds not typically associated with the radiolysis of oleic or linoleic fatty acids. To trace back the origin of these volatiles in the raw and irradiated almond samples it was imminent to observe the impact of irradiation onto the second most abundant component of almonds, proteins. It is important to remember that when food is irradiated, chemical changes occur as a result of direct action on carbohydrates, fats, proteins, and by indirect action of water radiolysis. In a multicomponent matrix such as food, constituents exert certain degree of protection among each other, thereby reducing the direct effect on any individual component (Mollins 2001). When proteins are subjected to ionizing radiation,

chain fracture is one of the main occurrences. As a consequence of the amino acid chain breakage, at least one of the resulting chains will endure the loss of an amino group, or a carboxyl group, or the formation of a carbonyl group (Josephson and others 1989). The main irradiation by-products of peptides are ammonia, fatty acids, and diamino acids (Molins 2001).

Aromatic amino acids (phenylalanine, tyrosine and tryptophan) are most likely to be affected by irradiation because the  $\cdot\text{OH}$  radicals produced during irradiation can easily become attached to the  $\Pi$ -electron of the aromatic ring (Jovanovic and Josimovic 1992). Benzene and toluene are formed from the breakdown of phenylalanine, (Josephson 1989). In the present study, benzene, 1,3,5-trimethyl benzene, and ethyl toluene were found in the chromatogram of the tested almonds.

Sulfur containing amino acids (methionine and cysteine) are also highly sensitive to irradiation due to the presence of the disulfide group. Exposing the disulfide group to ionizing energy can result in the oxidation, and further degradation of the SH and -S-S- groups. The radiolysis of methionine can lead to the formation of homocysteine and methyl mercaptan. The radiolysis of cysteine can result in the production of stable products such as hydrogen, hydrogen sulfide, alanine and cystine. The production of hydrogen sulfide and methyl mercaptan are a concern due to their known off-odors and off-flavors (Molins 2001).

In a study by Jo and Ahn (2000) four sulfur-containing compounds, methanethiol, 2-propenal, dimethyldisulfide, dimethyltrisulfide, 3-methylthiopropenal, were produced after irradiating an oil emulsion containing methionine. These

compounds have been suggested as the main off-odor generators in chicken meat (Patterson and Stevenson 1995). Similarly, the major source of off-odor production in irradiated almonds could be sulfur-containing amino acids via the radiolytic degradation of their side chains. In the present study, there were 39 volatiles identified in the headspace of irradiated almonds, 18% of them were traced back to the radiolysis of amino acids.

#### *Aromatrax<sup>®</sup> results*

As soon as the raw and irradiated almonds were received by the specialized analytical third party laboratory, an overall odor evaluation of the samples was performed. The raw almonds had a normal almond flavor, with maybe a floral or soapy undertone. For the irradiated samples, the descriptors included meaty, medicinal, old rancid oil, stale, old, smoky, vegetable broth and cardboard.

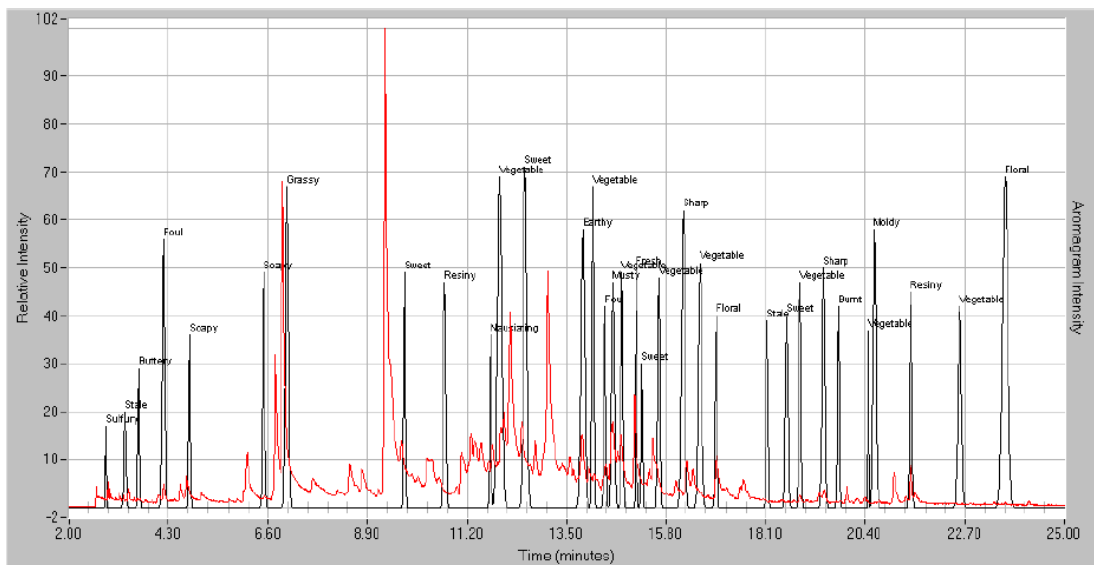
Aromagrams are the graphical representation of a sample's odor intensity versus retention time in the GC/MS chromatogram. Simultaneously to the corresponding GC/MS chromatograms, the aromagrams of raw and irradiated almonds were recorded using the AromaTrax<sup>®</sup> GC/MS/Olfactometry integrated system. For each aroma detected at the sniff port, the analyst recorded a descriptor and its relative intensity. The odor intensity has a qualitative meaning that helps evaluate the odor impact on the total odor of the sample. For the raw almonds, more than 30 odor notes were perceived, whereas for the irradiated almonds, over 40 odor notes were detected. The intensity for most of the odors was only moderately strong. The odor intensity is a qualitative number

based on the analyst's assessment of each compound as it was detected at the sniff port. These aroma characters were matched with the corresponding chromatographic peaks in Figs. 29-30 for raw and irradiated almonds, respectively. Figures 29 and 30 show the overlay of the aromagram with the corresponding chromatogram. Aroma characters and match compounds are summarized in Table 8, which lists the retention time, the odor character, the tentative identification and the odor intensity for each aroma note. A sulfury, foul odor character was identified and the tentative chemical identification pointed to methyl mercaptan. Coincidentally, methyl mercaptan is one of the breakdown products of the amino acid cysteine (Jo and Ahn 2000).

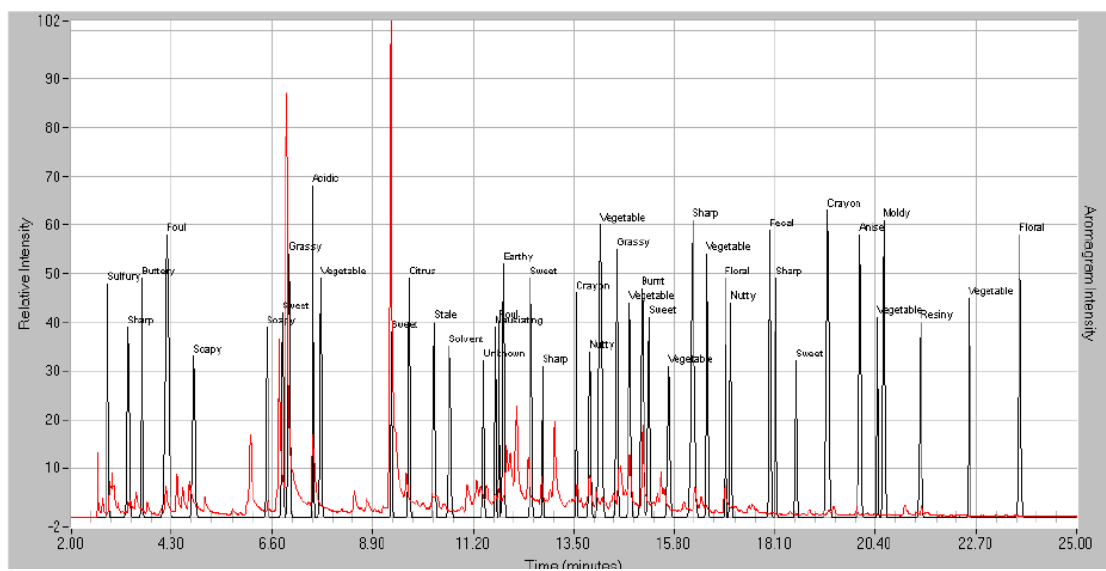
According to these instrumental analyses, the minor differences in the aroma/odor profile, and taste analysis of raw and irradiated almonds indicated that the differences between raw and irradiated samples are extremely subtle. The odor intensity for many of the compounds had only a moderate impact, with no single compound giving a strong dominating characteristic odor. The current evidence suggests that the radiolytic products of almond's components, such as lipids and proteins, may be the major sources of off-odor volatiles in irradiated almonds.

As a final note, it is important to mention that mechanical damage to the cellular fat compartments plays an important role in the stability of seeds. The breakage of the fat cells leads the cellular contents to enter in contact with O<sub>2</sub> (Fennema 2000). In the case of the present work, the almonds fat cells were not severed, maintaining the oils contained and away from environmental O<sub>2</sub>, which may have been beneficial to the almonds oil stability.





**Figure 29-Aromagram<sup>®</sup> and chromatogram corresponding to raw almonds.**



**Figure 30-Aromagram<sup>®</sup> and chromatogram of irradiated almonds.**

**Table 8-Aroma profile of raw and irradiated almonds with the retention time, odor character, tentative identification and odor intensity for each aroma note.**

Retention time	Odor character	Tentative identification	Odor Intensity	
			Raw	Irradiated
2.81	Sulfury, foul	Methyl mercaptan	101	335
3.25	Sharp, stale, earthy	propanal	259	506
3.6	Buttery, sweet	diacetyl	289	293
4.09	Foul, early, solvent	3-methyl-butanal	894	1215
4.74	Soapy, solvent	Pentanal	359	461
6.43	Soapy, solvent		733	428
6.79	Sweet, citrus	Ethyl butyrate		419
6.94	Grassy, green, herbaceous	Hexanal	1203	646
7.51	Acidic	Acetic acid		339
7.68	Vegetable			489
9.30	Sweet, fruity	n-hexanol		265
9.69	Citrus, fruity, sweet	n-heptanal	489	440
10.26	Stale	2-butoxy ethanol		439
10.59	Solvent, resinous, sharp		797	524
11.40	Unknown			255
11.67	Nauseating	Isolvaleric acid	395	272
11.78	Foul	Dimethyl trisulfide		159
11.84	Earthy, musty, vegetable, stale,		1859	519
12.47	Sweet, fresh, fruity, citrus	Octanal	1630	587
12.76	Sharp, rancid	Valeric acid		185
13.53	Crayon			229
13.81	Nutty, earthy, musty	Octenal	1331	373
14.03	Vegetable, herbaceous, nutty	Propyl toluene isomer	802	1018
14.32	Foul, stale		545	
14.44	Grassy, green, herbaceous			603
14.49	Must, moldy, mushroom	Ethyl styrene	797	
15.07	Fresh		449	
15.17	Sweet, citrus, fresh	Nonanal	269	450
15.60	Vegetable, nutty, stale		670	433
16.11	Sharp, stale, solvent	Guaiacol	1361	1339
16.50	Vegetable, green, fresh		1120	485
16.94	Floral, rose	1-nonanol	519	342
17.05	Nutty, meaty			395
17.94	Fecal	p-cresol		589
18.09	Sharp, stale, vegetable	2, 4-nonadienal	506	391
18.55	Sweet, anise	Octanoic acid	638	287
18.83	Vegetable	2-decenal	656	
19.24	Crayon			943
19.35	Sharp, spicy		848	
19.73	Burnt, cardboard		461	
20.00	Anise, sweet			694
20.43	Vegetable, nutty	Nonanoic acid	184	245
20.54	Moldy, mushroom, musty	Trichloroanisole	1042	669
21.36	Resinous	n-butyl butyrate	718	559
22.46	Vegetable, savory	Alpha-ionone	1006	359
23.63	Floral, rose	Pentadecane	2342	868

Additionally, phenolic compounds in the almonds skin, such as isorhamnetin rutinoside, isorhamnetin glucoside, kaempferol rutinoside, kaempferol glucoside, quercetin, naringenin, catechin, protocatechuic acid, vanillic acid and a benzoic acid derivative, may have played an important role in the delay of fat oxidation, since the main antioxidant mode of action of phenols is by scavenging free radicals (Harrison and Were 2007).

## CONCLUSIONS

The method developed to adjust the  $A_w$  of almonds, or a low moisture product, after the inoculation of a bacterial suspension is a breakthrough achievement that may be seen in the future work of the scientific community. At the truthful  $A_w$  level, electron beam irradiation, blanching and oil roasting technologies were capable of conferring the mandated 4-log reduction of *Salmonella* in almonds. Irradiation, blanching and oil roasting D-values for *S. Enteritidis* PT 30 on Nonpareil almonds was found to be 0.90 kGy, 15 and 13 s, and the D-values of *S. Senftenberg* were not significantly different ( $P > 0.05$ ). Thus, this study showed that current blanching and oil roasting commercial interventions are adequate processes to control *S. Enteritidis* PT 30 and, including the worst-case-scenario heat-resistant, *S. Senftenberg*.

The escalation of e-beam to be implemented as a commercial pathogen intervention was evaluated by Monte Carlo simulations and experimental measurements. Other food industries interested in the application of e-beam have faced the challenge of dose uniformity. The geometry and dimensions of the foods have a directly impact in the dose uniformity ratio (DUR), and consequently the risk of over or under-processing. In this work, the DUR of the current packaging configuration was within the commercial acceptability range. However, the reengineering of the almond's bulk packaging is an opportunity to reduce the DUR, and avoid the over-processing of some sections in the current package.

Consumer sensory data indicated that commercially e-beam irradiated almonds had no significant differences against blanched almonds at the overall like parameter. However, e-beam irradiated almonds had lower acceptability in terms of texture level, bitterness, and rancidity levels when compared to commercially blanched and oil roasted almonds. This research determined the safety threshold dose for almonds using electron beam irradiation. However, it is apparent that there is a discrepancy between the minimum necessary dose for safety and the maximum dose allowable by the consumer.

When consumers were asked to rank seven potential almond pasteurization technologies based on their personal acceptability, electronic pasteurization was the second most acceptable technology after dry roasting. This finding suggests that, to these days, consumers are more accepting of the use of e-beam irradiation in the food supply. In such scenario, irradiation may be an excellent option as an effective step in the pasteurization of almonds.

Quality indicators of irradiated, blanched and oil roasted almonds were tested during an ASLT for 4 wk at 50 °C. Such conditions represented half the expected commercial shelf life of almonds. However, the results obtained from the %FFA, PV and TBARs analysis were limited at providing sufficient sensitivity when compared to responses by the consumers. Therefore, a chemical test that accurately correlates with undesirable sensory parameters is an opportunity that the ABC may be interested in further exploring.

Finally, the GC/MS and Aromagram<sup>®</sup> analysis provided evidence to conclude that the application of an irradiation treatment increases the oxidation of the lipid

fraction of almonds. However, it was observed that the lipids are not the only component responsible for the off odors/flavors found in the irradiated almonds. The presence of amino acids breakdown products, together with lipid oxidation compounds, provide answers to the consumer's perceptions of the uncommon flavor profile found in irradiated almonds.

The ultimate objective of the present work was achieved by demonstrating the capability of e-beam irradiation in ensuring the safety of almonds, regardless of the low *A<sub>w</sub>* challenge posed by the innate characteristic of the product. The application of electron beam irradiation to control *Salmonella* from almonds may become a feasible alternative treatment if used as part of a hurdle, while acknowledging that the last word is given by the consumers.

## REFERENCES

Almond Board of California [ABC]. 2008. The food safety program & almond pasteurization. Modesto, CA. [Accessed 2010 August 1]. Available from: <http://www.almondboard.com/Handlers/FoodQualitySafety/Pasteurization/Pages/Default.aspx>.

Almond Board of California [ABC]. 2009a. Almond almanac. Modesto, CA. [Accessed 2010 January 2010]. Available from: <http://www.almondboard.com/AboutTheAlmondBoard/Pages/ResearchStats.aspx>.

Almond Board of California [ABC]. 2009b. Good agricultural practices. Modesto, CA. [Accessed 2010 January 2010]. Available from: <http://www.almondboard.com/AboutTheAlmondBoard/Pages/ResearchStats.aspx>.

Almond Board of California [ABC]. 2010a. Almonds as ingredients. Modesto, CA. [Accessed 2010 August 1 ]. Available from: <http://www.almondboard.com/FoodProfessionals/AlmondsInFood/AlmondsAsIngredients/Pages/default.aspx>.

Almond Board of California [ABC]. 2010b. Orchard management. Modesto CA. [Accessed 2010 August 1]. Available from: <http://www.almondboard.com/Growers/OrchardManagement/Pages/default.aspx>.

Almond Board of California [ABC]. 2010c. The team behind every crunch. Modesto, CA. [Accessed 2010 August 1]. Available from: <http://www.almondboard.com/AboutTheAlmondBoard/Pages/default.aspx>.



Almond Board of California [ABC]. 2010d. Validation guidelines. Modesto, CA. [Accessed 2010 August 1]. Available from:  
<http://www.almondboard.com/Handlers/FoodQualitySafety/Pasteurization/PasteurizationProgram/ValidationGuidelines/Pages/Default.aspx>.

Ahu DU, Lee EJ. 2006. Mechanisms and prevention of quality changes in meat by irradiation. In: Sommers CH, Fan X, editors. Food irradiation research and technology. Ames, IA: Blackwell Publishing Professional p 127-142

Agricultural Marketing Service [AMS]. 2010. Marketing Orders. USDA [Accessed 2010 August 1]. Available from:  
<http://www.ams.usda.gov/AMSV1.0/ams.fetchTemplateData.do?template=TemplateN&page=FVMarketingOrderIndexAlmonds>.

American Oil Chemists' Society [AOCS]. 1973. Official methods and recommended practices of the American oil chemists' society. Urbana, IL: American Oil Chemists' Society.

Arvanitoyannis IS, Stratakos AC, Tsarouhas P. 2009. Irradiation applications in vegetables and fruits: A Review. *Crc Cr Rev Food Sci* 49:427-62.

Bang IS, Frye JG, McClelland M, Velayudhan J, Fang FC. 2005. Alternative sigma factor interactions in *Salmonella*:  $\sigma^E$  and  $\sigma^H$  promote antioxidant defences by enhancing  $\sigma^S$  levels. *Mol Microbiol* 56:811-23.

Bari ML, Nei D, Sotome I, Nishina I, Isobe S and Kawamoto S. 2009. Effectiveness of sanitizers, dry heat, hot water, and gas catalytic infrared heat treatments to inactivate *Salmonella* on almonds. *Foodborne Patog Dis* 8:953-8.

Bari L, Nei D, Sotome I, Nishina IY, Hayakawa F, Isobe S, Kawamoto S. 2010. Effectiveness of superheated steam and gas catalytic infrared heat treatments to inactivate *Salmonella* on raw almonds. *Foodborne Patog Dis* 7:845-50.

Battista J. R.(1996). AGAINST ALL ODDS: The Survival Strategies of *Deinococcus radiodurans* *Annu. Rev. Microbiol.* 51:203–24

Berk Z. 2009. *Food process engineering and technology*. I. Burlington, MA: Academic Press. 605 p.

Beuchat LR. 1987. Influence of water activity on sporulation, germination, outgrowth, and toxin production. In: Rockland LB, Beuchat LR, editors. *Water activity: theory and applications to food*. Chicago, IL: IFT Basic Symposium Series. P 137-152.

Beuchat LR, Heaton EK. 1975. *Salmonella* survival on pecans as influenced by processing and storage conditions. *Appl Microbiology* 29:795-801.

Bianchi AA, Baneyx F. 1999. Hyperosmotic shock induces the  $\sigma^{32}$  and  $\sigma^E$  stress regulons of *Escherichia coli*. *Mol Microbiol* 34:1029-38.

Blank G, Cumming R. 2001. Irradiation. In: Eskin NAM, Robinson DS, editors. *Food shelf life stability*. I. Boca Raton, FL: CRC Press LLC. P 87-128.

Brandl MT, Pan Z, Huynh S, Zhu Yi, McHugh T. 2008. Reduction of *Salmonella* Enteritidis population sizes on almond kernels with infrared heat. *J Food Prot* 71:897-902.

Buransompob A, Tang J, Mao R, Swanson BG. 2003. Rancidity of walnuts and almonds affected by short time heat treatments for insect control. *J Food Process Pres* 27:445-64.

Byrd JA, Corrier DE, De Loach JR, Nisbet DJ. 1997. Comparison of drag-swab environmental protocols for the isolation of *Salmonella* in poultry houses. *Avian Dis* 41:709-13.

Cabiscol E, Tamarit J, Ros J. 2000. Oxidative stress in bacteria and protein damage by reactive oxygen species. *Int Microbiol* 3:3-8.

Cabrera Diaz E. 2007. Characterization and evaluation of *E. coli* biotype I strains for use as surrogates for enteric pathogens in validation of beef carcass interventions [dissertation]. College Station, Tex.: Texas A&M University. 175 p. Available from: Repository Texas A&M University, College Station, Tex. [dissertation].

Calligaris S, Manzocco L, Conte L, Nicoli M. 2004. Application of a modified arrhenius equation for the evaluation of oxidation rate of sunflower oil at subzero temperatures. *J Food Sci* 69:E361-E6.

Centers for Disease Control and Prevention [CDC]. 2004. Outbreak of *Salmonella* serotype Enteritidis infections associated with raw almonds-- United States and Canada, 2003-2004. *MMWR* 53:484-7. [Accessed 2007 Septiembre 19]. Available from: <http://cdc.gov/mmwr/preview/mmwrhtml/mm53d604a1.htm>.

Centers for Disease Control and Prevention [CDC]. 2009. Multistate outbreak of *Salmonella* infections associated with peanut butter and peanut butter--Containing products --- United States, 2008--2009. *MMWR* 58:85-90. [Accessed 2010 Aug 1]. Available from: <http://www.cdc.gov/mmwr/preview/mmwrhtml/mm5804a4.htm>.

Centers for Disease Control and Prevention [CDC]. 2010. *Salmonella*. General information.[Accessed 2010 August 2010]. Available from:

<http://www.cdc.gov/salmonella/general/index.html>.

Centers for Disease Control and Prevention [CDC]. 2010. *Salmonella* outbreaks. [Accessed 2010 August 2010]. Available from:

<http://www.cdc.gov/salmonella/outbreaks.html>.

Chang SS, Han A, Reyes-De-Corcuera J, Powers J, Kang DH. 2010. Evaluation of steam pasteurization in controlling *Salmonella* serotype Enteritidis on raw almond surfaces. *Lett Appl Microbiol* 50:393-8.

Chen CY, Lapsley K, Blumberg J. 2006. A nutrition and health perspective on almonds. *J Sci Food Agr* 86:2245-50.

ConAgra Foods, Inc [ConAgra]. 2007. ConAgra Foods announces the renovation of its peanut butter plant and enhanced food safety measures. Omaha, NE: [Accessed 2010 August 1]. Available from:

<http://investor.conagrafoods.com/phoenix.zhtml?c=202310&p=irolnewsArticle&ID=1008473&highlight=>.

Corry JEL. 1974. The effect of sugars and polyols on the heat resistance of salmonellae. *J Appl Microbiol* 37:31-43.

Csonka LN. 1981. Proline over-production results in enhanced osmotolerance in *Salmonella* Typhimurium. *Mol Gen Genet* 182:82-6.

D'Aoust J-Y. 1977. *Salmonella* and the chocolate industry. A review. *J Food Prot* 40:718-27.

D'Aoust J-Y. 1997. *Salmonella*. In: Doyle MP, Beuchat LR, Montville TE, editors. Food Microbiology: Fundamentals and frontiers. 2<sup>nd</sup> edition. Washington D.C.: American Society for Microbiology. p 187-236.

D'Aoust J-Y. 2000. *Salmonella*. In: Mund BL, Baird-Parker TC, Gould GW, editors. The microbiological safety and quality of food. Gaithersburg, Maryland: Aspen Publishers Inc. p 1233-1299.

Danyluk MD, Brandl MT, Harris LJ. 2008a. Migration of *Salmonella* Enteritidis phage type 30 through almond hulls and shells. J Food Protect 71:397-401.

Danyluk MD, Harris LJ, Schafner DW. 2006. Monte carlo simulations assessing the risk of salmonellosis from consumption of almonds. J Food Prot 69:1594-9.

Danyluk MD, Jones TM, Abd SJ, Schlitt-Dittrich F, Jacobs M, Harris LJ. 2007. Prevalence and amounts of *Salmonella* found on raw California almonds. J Food Prot 70:820-7.

Danyluk MD, Nozawa-Inoue M, Hristova K, Scow K, Lampinen B, Harris LJ. 2008b. Survival and growth of *Salmonella* Enteritidis PT 30 in almond orchard soils. J Appl Microbiol 104:1391-9.

Danyluk MD, Uesugi AR and Harris LJ. 2005. Survival of *Salmonella* Enteritidis PT 30 on inoculated almonds after commercial fumigation with propylene oxide. J Food Prot 68:1613-22.

Darwin KH, Miller VL. 1999. Molecular basis of the interaction of *Salmonella* with the intestinal mucosa. Clin Microbiol Rev 12:405-28.

Deng S, Ruan R, Mok CK, Huang G, Lin X, Chen P. 2007. Inactivation of *Escherichia coli* on almonds using nonthermal plasma. J Food Sci 72:M62-M6.

Blue Diamond. 2010. Understanding and maximizing shelf life of almonds. Blue diamond growers [Accessed 2010 September 4 ]. Available from: <http://www.bluediamond.com/index.cfm?navId=131>.

Dickson J. 2001. Radiation Inactivation of Microorganisms In: Molins R. Food irradiation: principles and application. Danvers MA: John Wiley & Sons, Inc. p 23-36.

Doesburg JJ, Lamprecht EC, Elliott M. 1970. Death rates of salmonellae in fishmeals with different water activities II.—During heat treatment. J Sci Food Agr 21:636-40.

Du W-X, Harris LJ. 2005 March. Survival of *Salmonella* Enteritidis PT 30 on inoculated almonds after treatment in hot oil. University of California, Davis, Calif.: Report to the FDA-CFSAN on behalf of the Almond Board of California. 17 p.

Dupy H.P., Rayner E.T., Wadsworth J.I. 1973. Correlations of flavor score with volatiles of vegetable oils. J Amer Oil Chem Soc 10:628-31

Dupuy H.P., Fore S.P., Goldblatt L.A. 1973. Direct gas chromatographic examination of volatiles in salad. J Amer Oil Chem Soc 9:340-42

Faid M, Bakhy K, Anchad M, Tantaoui-Elaraki A. 1995. Almond paste: physicochemical and microbiological characterization and preservation with sorbic acid and cinnamon. J Food Prot 58:547-50.

Food and Drug Administration. 2009 [FDA]. Food irradiation: A safe measure. [Accessed 2009 January 18]. Available from:

<http://www.fda.gov/opacom/catalog/irradbro.html>.

Food and Drug Administration Administration [FDA]. 2010a. *Salmonella* spp.[Accessed 2010 Aug 1]. Available from:

<http://www.fda.gov/food/foodsafety/foodborneillness/foodborneillnessfoodbornepathogensnaturaltoxins/badbugbook/ucm069966.htm>.

Food and Drug Administration Administration [FDA]. 2010b. Pistachio and other pistachio containing products recall list.[Accessed 2010 Aug 1]. Available from:

<http://www.fda.gov/Safety/Recalls/MajorProductRecalls/Pistachio/Update/default.htm>.

Food and Drug Administration Administration [FDA]. 2011. Guidance for industry: A food labeling guide. Appendix D: Qualified health claims .[Accessed 2011 Nov 11]. Available from:

<http://www.fda.gov/Food/GuidanceComplianceRegulatoryInformation/GuidanceDocuments/FoodLabelingNutrition/FoodLabelingGuide/ucm064923.htm>

Fellows P. 2002. Blanching. In: Fellows P, editor. Food processing technology: principles and practice. Boca Raton, FL: CRC Press Taylor & Francis Group. p 233-240.

Fennema OR. 2000. Agua y hielo. In: Fennema OR, editor. Química de los alimentos. 2nd. Zaragoza, España: Acribia, S.A. p 19-109.

Fletcher SA, Csonka LN. 1998. Characterization of the induction of increased thermotolerance by high osmolarity in *Salmonella*. Food Microbiol 15:307-17.

Fletcher SA, Rhodes D, Csonka LN. 2001. Analysis of the effects of osmoprotectants on the high osmolality-dependent induction of increased thermotolerance in *Salmonella typhimurium*. *Food Microbiol* 18:345-54.

Forbes BA, Sahm DF, Weissfeld AS. 2002. *Diagnostic microbiology*. 11th. St. Louis MO: Mosby, Inc. 1069 p.

Galán JE. 2001. *Salmonella* interactions with host cells: Type III secretion at work. *Annu Rev Cell Dev Biol* 17:53-86.

García-Pascual P, Mateos M, Carbonell V, Salazar DM. 2003. Influence of storage conditions on the quality of shelled and roasted almonds. *Biosyst Eng* 84:201-9.

Georgopoulos C. 1992. The emergence of the chaperone machines. *Trends Biochem Sci* 17:295-9.

Goepfert JM, Iskander IK, Amundson CH. 1970. Relation of the heat resistance of salmonellae to the water activity of the environment. *Appl Microbiol* 19:429-33.

Goodridge LD, Willford J, Kalchayanand N. 2006. Destruction of *Salmonella* Enteritidis inoculated onto raw almonds by high hydrostatic pressure. *Food Res Int* 39:408-12.

Gunasekera TS, Csonka LN, Paliy O. 2008. Genome-wide transcriptional responses of *Escherichia coli* K-12 to continuous osmotic and heat stresses. *J Bacteriol* 190:3712-20.

Harris LJ, Danyluk MD, Beuchat LR, Palumbo M. 2009. Outbreaks associated with the consumption of tree nuts, peanuts, and sesame seeds. University of California at



Davis. [Accessed 2010 Aug 1]. Available from:

<http://ucfoodsafety.ucdavis.edu/files/26459.pdf>.

Harrison K, Were L.M. 2007. Effect of gamma irradiation on total phenolic content yield and antioxidant capacity of almond skin extracts. *Food Chem* 102:932-37.

Hartl FU, Hayer-Hartl M. 2002. Molecular chaperones in the cytosol: from nascent chain to folded protein. *Science* 295:1852-8.

Hayes DJ, Murano EA, Murano PS, Olson DG, Sapp SG. 1995. Food irradiation - A Sourcebook. I. Ames, IA: Iowa State University Press. 136 p.

Henry NG, Bayne HG, Garibaldi JA. 1969. Heat resistance of *Salmonella*: the uniqueness of *Salmonella* senftenberg 775W. *App Micro* 17:78-82

Health Protection Agency [HPA]. 2010. A national outbreak of *Salmonella* Montevideo infection June 2006. [Accessed 2010 Aug 1]. Available from: [http://www.hpa.org.uk/web/HPAwebFile/HPAweb\\_C/1207293973143](http://www.hpa.org.uk/web/HPAwebFile/HPAweb_C/1207293973143).

Hollis J, Mattes R. 2007. Effect of chronic consumption of almonds on body weight in healthy humans. *Br J Nutr* 98:651-6.

Isaacs S, Aramini J, Ciebin B, Farrar JA, Ahmed R, Middleton D, Chandran AU, Harris J, Howes M, Chan E, Pichette AS, Campbell K, Gupta A, Lior LY, Pearce M, Clark C, Rodgers F, Jamieson F, Brophy I, Ellis A. 2005. An international outbreak of salmonellosis associated with raw almonds contaminated with a rare phage type of *Salmonella* Enteritidis. *J Food Prot* 68:191-8.

Jay JM, Loessner MJ, Golden DA, editors. 2005. Modern food microbiology. New York NY: Springer Science. 790 p.

Jeong S, Marks BP, Orta-Ramirez A. 2009. Thermal inactivation kinetics for *Salmonella* Enteritidis PT30 on almonds subjected to moist-air convection heating. *J Food Prot* 72:1602-9.

Jones BD, Falkow S. 1996. SALMONELLOSIS: host immune responses and bacterial virulence determinants. *Annu Rev Immunol* 14:533-61.

Juven BJ, Cox NA, Bailey JS, Thomson JE, Charles OW, Shutze JV. 1984. Survival of *Salmonella* in dry food and feed. *J Food Prot* 47:445-8.

Kaspar CW, Tamplin ML. 1993. Effects of temperature and salinity on the survival of *Vibrio vulnificus* in seawater and shellfish. *Appl Environ Microbiol* 59:2425-9.

Kester DE, Ross NW. 1996. History. In: Micke WC, editor. Almond production manual. Oakland, CA: University of California. Division of Agriculture and Natural Resources. p 1-2.

King AJ, Miller M, Eldridge L. 1970. Almond harvesting, processing, and microbial flora. *Appl Microbiol* 20:208-14.

Kingsley RA, Bäumler AJ. 2000. Host adaptation and the emergence of infectious disease: the *Salmonella* paradigm. *Mol Microbiol* 36:1006-14.

Kingston DJ. 1981. A comparison of culturing drag swabs and litter for identification of infections with *Salmonella* spp. in commercial chicken flocks. *Avian Dis* 25:513-6.

Kirk MD, Little CL, Lem M, Fyfe M, Genobile D, Tan A, Threlfall J, Paccagnella A, Lightfoot D, Lyi H, McIntyre L, Ward L, Brown DJ, Surnam S, Fisher

IST. 2004. An outbreak due to peanuts in their shell caused by *Salmonella* Enterica serotypes Stanley and Newport: sharing molecular information to solve international outbreaks. *Epidemiol Infect* 132:571-7.

Knorr D, Heinz V, Luscher C. 2007. Thermal processing of foods: Technological aspects. In: deutsche forschungsgemeinschaft. Thermal processing of food: potential health benefits and risks. Germany: Wiley-VCH Verlag GmbH & Co. KGaA. p 17-25.

Kodad O, Socias I Company R. 2008. Variability of oil content and of major fatty acid composition in almond (*Prunus amygdalus* Batsch) and its relationship with kernel quality. *J Agric Food Chem* 56:4096-101.

Komitopoulou E, Peñaloza W. 2009. Fate of *Salmonella* in dry confectionery raw materials. *J Appl Microbiol* 106:1892-900.

Labuza TP, Schmidl MK. 1985. Accelerated shelf-life testing of foods. *Food Technol* 57-64.

Lapsley KG, Huang G. 2004. Health benefits of almonds. *AACC* 49:6.

Lee S-Y, OH S-W, Chung H-J, Reyes-de-Corcuera JI, Powers JR, Kang D-H. 2006. Reduction of *Salmonella* enterica serovar Enteritidis on the surface of raw shelled almonds by exposure to steam. *J Food Prot* 69:591-5.

Lee SY, Guinard JX, Krochta JM. 2003. Relating sensory and instrumental data to conduct an accelerated shelf-life testing of whey-protein-coated peanuts. In: Cadwallader KR, Weenen H, editors. Freshness and shelf life of foods. Washington, DC: American Chemical Society. p 175-187.

Lehmacher A, Bockemühl J, Aleksic S. 1995. Nationwide outbreak of human salmonellosis in Germany due to contaminated paprika and paprika-powdered potato chips. *Epidemiol Infect* 115:501-11.

Liu TS, Snoeyenbos GH and Carlson VL. 1969. Thermal resistance of *Salmonella* Senftenberg 775W in dry animal feeds. *Avian Dis* 13:611-31.

Mandalari G, Nueno-Palop C, Bisignano G, Wickham MSJ, Narbad A. 2008. Potential prebiotic properties of almond (*Amygdalus communis* L.) seeds. *Appl Environ Microb* 74:4264-70.

Mattick KL, Jorgensen F, Legan JD, Cole MB, Porter J, Lappin-Scott HM, Humphrey TJ. 2000a. Survival and filamentation of *Salmonella* enterica serovar Enteritidis PT4 and *Salmonella* enterica serovar Typhimurium DT104 at low water activity. *Appl Environ Microb* 66:1274-9.

Mattick KL, Jorgensen F, Legan JD, Lappin-Scott HM, Humphrey TJ. 2000b. Habituation of *Salmonella* spp. at reduced water activity and its effect on heat tolerance. *Appl Environ Microb* 66:4921-5.

Mattick KL, Jorgensen F, Wang P, Pound J, Vandeven MH, Ward LR, Legan JD, Lappin-Scott HM, Humphrey TJ. 2001. Effect of challenge temperature and solute type on heat tolerance of *Salmonella* serovars at low water activity. *Appl Environ Microb* 67:4128-36.

McDonough FE, Hargrove RE. 1968. Heat resistance of *Salmonella* in dried milk. *J Dairy Sci* 51:1587-91.

Mexis SF, Badeka AV, Chouliara E, Riganakos KA, Kontominas MG. 2009. Effect of  $\gamma$ -irradiation on the physicochemical and sensory properties of raw unpeeled almond kernels (*Prunus dulcis*). *Innov Food Sci Emerg* 10:87-92.

Micke WC. 1996. Almond production manual. Oakland, CA: University of California Division of Agriculture and Natural Resources. 289 p.

Miller RB. 2005. Electronic irradiation of foods. New York NY: Springer Science. 295 p.

Mizrahi S. 2000. Accelerated shelf-life test. In: Kilcast D, Subramaniam P, editors. The stability and shelf-life of food. Boca Raton, FL: CRC Press, LLC. p 107-128.

Morehouse KM, and Komolprasert V. 2004. Irradiation of food and packaging: an overview In: Irradiation of food and packaging. ACS Symposium series 875. p 1-11.

Muller LL, Hjertqvist M, Payne L, Pettersson H, Olsson A, Plym-Forshell L, Andersson. Y. 2007. Cluster of *Salmonella* Enteritidis in Sweden 2005-2006 -suspected source almonds. *Euro Surveil* 12:E9-10

Narvaiz P, Lescano G, Kairiyama E. 1992. Irradiation of almonds and cashew nuts. *Lebensm Wiss Technol* 25:232-5.

Navarro S, Isikber AA, Finkelman S, Rindner M, Azrieli A, Dias R. 2004. Effectiveness of short exposures of propylene oxide alone and in combination with low pressure or carbon dioxide against *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae). *J Stored Prod Res* 40:197-205.

Nayga RM, Aiew W, Nichols JP. 2005. Information effects on consumers' willingness to purchase irradiated food products. *Rev Agr Econ* 27:37-48.

NG H, Bayne HG, Garibaldi JA. 1969. Heat resistance of *Salmonella*: the uniqueness of *Salmonella* Senftenberg 775 W. *Appl Microbiol* 17:78-82.

Niemira BA, Sommers CH, Boyd G. 2001. Irradiation inactivation of four *Salmonella* serotypes in orange juices with various turbidities. *J Food Prot* 64:614-7.

Ohl ME, Miller SI. 2001. *Salmonella*: A model for bacterial pathogenesis. *Annu Rev Med* 52:259-74.

Palekar MP. 2004. Attachment of *Salmonella* on cantaloupe and effect of electron beam irradiation on quality and safety of sliced cantaloupe. [DPhil dissertation]. College Station, TX: Texas A&M Univ. 99 p. Available from: Texas A&M University Repository. <http://repository.tamu.edu/>.

Pao S, Kalantari A, Huang G. 2006. Utilizing acidic sprays for eliminating *Salmonella enterica* on raw almonds. *J Food Sci* 71:M14-M9.

Patterson MF, Loaharanu P. 2000. Irradiation. In: Mund BL, Baird-Parker TC and Gould GW. *The microbiological safety and quality of food*. Gaithersburg, Maryland: Aspen Publishers Inc. p 65-100.

Peanut Corporation of America [PCA]. 2009. Peanut Corporation of America provides further information regarding recalled products. Lynchburg, VA: [Accessed Available from: [http://www.peanutcorp.com/pdf/2\\_20\\_09.pdf](http://www.peanutcorp.com/pdf/2_20_09.pdf)].

Phung OJ, Makanji SS, White CM, Coleman CI. 2009. Almonds have a neutral effect on serum lipid profiles: A meta-analysis of randomized trials. *J Am Diet Assoc* 109:865-73.

Prakash A, Johnson N and Foley D. 2006. Irradiation D values of *Salmonella* spp. in diced tomatoes dipped in 1% calcium chloride. *Foodborne Patog Dis* 4:84-8.

Prakash A, Lim FT, Duong C, Caporaso F and Foley D. 2010. The effects of ionizing irradiation on *Salmonella* inoculated on almonds and changes in sensory properties. *Radiat Phys Chem* 79:502-6.

Price-Carter M, Tingey J, Bobik TA, Roth JR. 2001. The alternative electron acceptor tetrathionate Supports B<sub>12</sub>-Dependent Anaerobic Growth of *Salmonella* enterica serovar Typhimurium on ethanolamine or 1,2-propanediol. *J Bacteriol* 183:2463-75.

Ragnarsson JO, Labuza TP. 1977. Accelerated shelf-life testing for oxidative rancidity in foods--A review. *Food Chem* 2:291-308.

Ramesh MN. 2007. Pasteurization and food preservation. In: Rahman MS, editor. *Handbook of food preservation*. 2nd edition. Boca Raton, FL: CRC Press Taylor & Francis Group. p 571-584.

Ribó J, Crusats J, El-Hachemi Z, Feliz M, Sanchez-Bel P, Romojaro F. 2004. High-resolution NMR of irradiated almonds. *JAOCS* 81:1029-33.

Riemann H. 1968. Effect of water activity on the heat resistance of *Salmonella* in "dry" materials. *Appl Microbiol* 16:1621-2.

Rodriguez O, Castell-Perez ME, Ekpanyaskun N, Moreira RG, Castillo A. 2006. Surrogates for validation of electron beam irradiation of foods. *Int J Food Microbiol* 110:117-22.

Rosengarten F. 1984. *The book of edible nuts*. New York NY: Walker and Company. 384 p.

Sánchez-Bel P, Martínez-Madrid MC, Egea I and Romojaro F. 2005. Oil quality and sensory evaluation of almond (*Prunus amygdalus*) stored after electron beam processing. *J Agric Food Chem* 53:2567-73.

Senesi E, Rizzolo A, Colombo C, Testoni A. 1996. Influence of preprocessing storage conditions on peeled almond quality. *Ital J Food Sci* 8:115-25.

Severini C, Gomes T, De Pilli T, Romani S, Massini R. 2000. Autoxidation of packed almonds as affected by Maillard reaction volatile compounds derived from roasting. *J Agric Food Chem* 48:4635-40.

Severini C, Pilli TD, Baiano A, Gomes T. 2003. Autoxidation of packed roasted almonds as affected by two packaging films. *J Food Process Pres* 27:321-35.

Shachar D, Yaron S. 2006. Heat tolerance of *Salmonella* enterica serovars Agona, Enteritidis, and Typhimurium in peanut butter. *J Food Prot* 69:2687-91.

Sherry AE, Patterson MF, Madden RH. 2004. Comparison of 40 *Salmonella* enterica serovars injured by thermal, high-pressure and irradiation stress. *J Appl Microbiol* 96:887-93.



Takeuchi A. 1966. Electron microscope studies of experimental *Salmonella* infection. I. Penetration into the intestinal epithelium by *Salmonella* Typhimurium. Am J Pathol 50:109-36.

Tamminga SK, Beumer RR, Kampelmacher EH, Leusden FMV. 1977. Survival of *Salmonella* Eastbourne and *Salmonella* Typhimurium in milk chocolate prepared with artificially contaminated milk powder. J Hyg (Lond) 79:333-7.

Tan C, Che Man Y, Selamat J, Yusoff M. 2001. Application of Arrhenius kinetics to evaluate oxidative stability in vegetable oils by isothermal differential scanning calorimetry. JAOCS 78:1133-8.

Taokis P, Labuza TP. 2000. Resumen: integración conceptual. In: Fennema OR, editor. Química de los alimentos. 2nd. Zaragoza, España: Acribia, S.A. p 1201-36.

Tazi S, Plantevin F, Falco CD, Puigserver A, Ajandouz EH. 2009. Effects of light, temperature and water activity on the kinetics of lipoxidation in almond-based products. Food Chem 115:958-64.

Torabian S, Haddad E, Rajaram S, Banta J, Sabaté J. 2009. Acute effect of nut consumption on plasma total polyphenols, antioxidant capacity and lipid peroxidation. J Hum Nutr Diet 22:64-71.

Turner JE. 2007. Interaction of electrons with matter. In: Atoms, radiation, and radiation protection. 3rd. Weinheim, Germany: Wiley-VCH. P 139-49.

Uesugi AR, Harris LJ. 2005 March. Survival of *Salmonella* Enteritidis PT 30 on inoculated almonds after treatment in hot water. University of California, Davis, Calif.:

Report to the FDA-CFSAN on behalf of the Almond Board of California. 18 p.

Department of Food Science and Technology, University of California Davis

Uesugi AR, Danyluk M, Mandrell RE, Harris LJ. 2007. Isolation of *Salmonella* Enteritidis phage type 30 from a single almond orchard over a 5-Year Period. J Food Prot 70:1784-9.

Uesugi AR, Danyluk MD, Harris L. 2006. Survival of *Salmonella* Enteritidis phage type 30 on inoculated almonds stored at 20, 4, 23, and 35°C. J Food Prot 69:1851-7.

Uesugi AR, Harris L. 2006. Growth of *Salmonella* Enteritidis Phage Type 30 in almond hull and shell slurries and survival in drying almond hulls. J Food Prot 69:712-8.

[USDA]. U.S. Department of Agriculture. 2009. USDA National nutrient database for standard reference. [Accessed 2009 January 19]. Available from: [http://www.nal.usda.gov/fnic/foodcomp/cgi-bin/list\\_nut\\_edit.pl](http://www.nal.usda.gov/fnic/foodcomp/cgi-bin/list_nut_edit.pl).

Uthman RS, Toma RB, Garcia R, Medora NP, Cunningham S. 1998. Lipid analyses of fumigated vs irradiated raw and roasted almonds. J Sci Food Agr 78:261-6.

Voet D, Voet JG, Pratt CW. 2006. Fundamentals of biochemistry - Life at the molecular level. 2<sup>nd</sup> edition. Danvers MA: John Wiley & Sons, Inc. 1130p.

A Correlated Fitness Landscape Describes Growth in Experimental Microbial Ecosystems: Initial Results by Frederik Vandecasteele, Frederik P. J. V, Thomas Hess, Ronald Crawford, 2004.

Waletzko P, Labuza TP. 1976. Accelerated shelf-life testing of an intermediate moisture food in air and in an oxygen-free atmosphere. J Food Sci 41:1338-44.

Wallis TS, Galyov EE. 2000. Molecular basis of *Salmonella*-induced enteritis. *Mol Microbiol* 36:997-1005.

Werber D, Dreesman J, Feil F, van Treeck U, Fell G, Ethelberg S, Hauri A, Roggentin P, Prager R, Fisher I, Behnke S, Bartelt E, Weise E, Ellis A, Siitonen A, Andersson Y, Tschape H, Kramer M, Ammon A. 2005. International outbreak of *Salmonella* Oranienburg due to German chocolate. *BMC Infect Dis* 5:7.

Willford J, Mendonca A and Goodridge LD. 2008. Water pressure effectively reduces *Salmonella* Enterica serovar Enteritidis on the surface of raw almonds. *J Food Prot* 71:825-9.

Zacheo G, Cappello MS, Gallo A, Santino A, Cappello AR. 2000. Changes associated with post-harvest ageing in almond seeds. *Lebensm Wiss Technol* 33:415-23.

**VITA**

Name: Mary Pia Cuervo Pliego

Address: Department of Animal Science  
Kleberg Center  
Texas A&M University  
College Station Tx., 77843-2471

Email Address: mpia10@hotmail.com

Education: B.S., Ingeniería en Industrias Alimentarias, Instituto Tecnológico y de Estudios Superiores de Monterrey, 2004  
M.S., Food Science and Technology, Texas A&M University, 2007  
Ph.D., Food Science and Technology, Texas A&M University, 2011.

Professional Memberships: Texas Association for Food Protection, since 2005  
International Association for Food Protection, since 2008